LEAF ANATOMY AND CHEMOTAXONOMY IN GNAPHALIINAE (INULEAE - COMPOSITAE)

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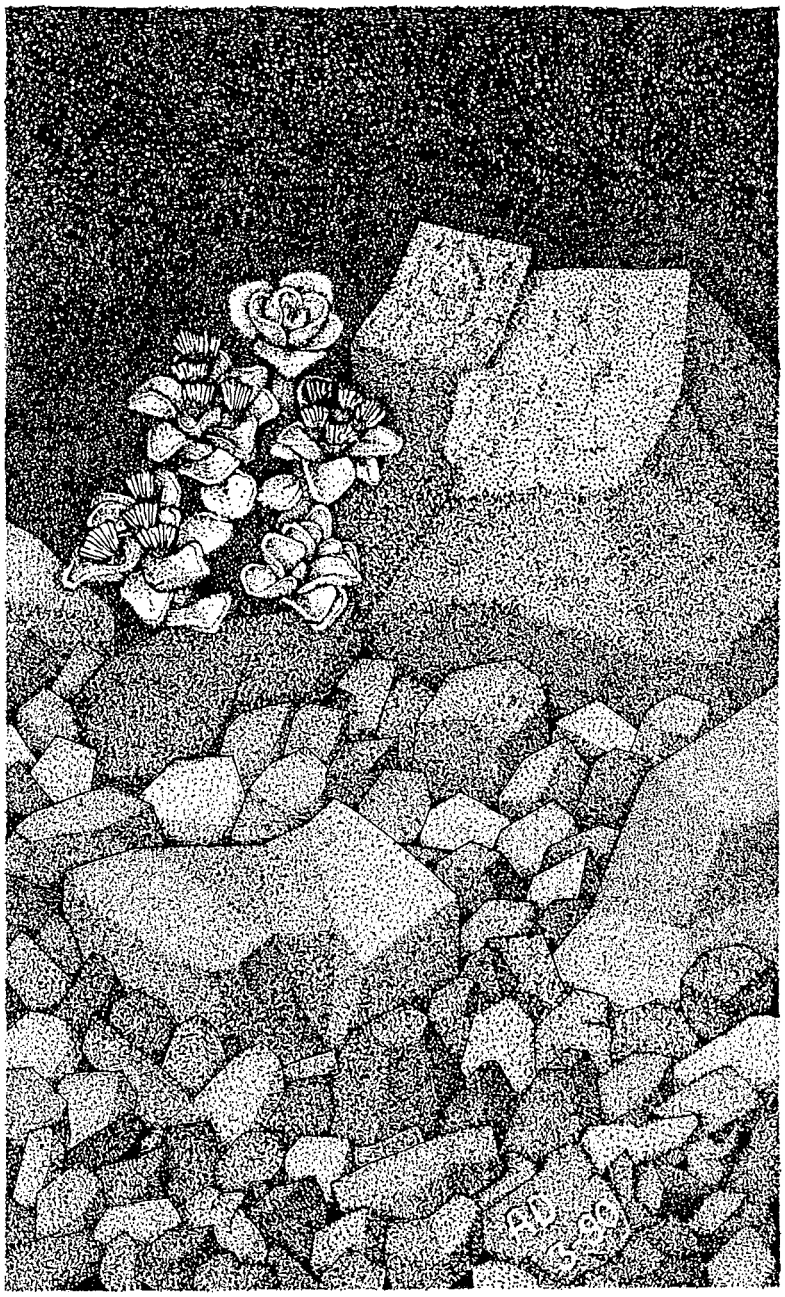

A new genus of scree daisy (Genus "Z")

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ABSTRACT

Leaf anatomical and chemotaxonomic studies were carried out on the subtribe Gnaphaliinae (Inuleae - Compositae) with the aim of clarifying the status and relationships of the New Zealand and Tasmanian taxa.

The leaf anatomy and flavonoid patterns of 48 species were compared. Data were analysed numerically and cladistically.

The Gnaphaliinae is a problematical group, especially in terms of generic boundaries. In the leaf anatomical and chemotaxonomic analyses, the New Zealand species of *Anaphalis* are similar to each other but quite different from a Himalayan representative (*Anaphalis triplinervis*). In the New Zealand species of *Helichrysum* the relationships of the two species of section *Xerochlaena* are unresolved but there is evidence that the New Zealand species of section *Ozothamnus* should be treated separately from *Helichrysum*. The New Zealand species of *Cassinia* are very similar to the Tasmanian species of *Helichrysum* sect. *Ozothamnus* but not to the Tasmanian species of *Cassinia*. *Ewartia sinclairii* of New Zealand is very different from the Tasmanian species of *Ewartia*. The exclusion of the anaphalioid species of *Gnaphalium* and of *Gnaphalium luteo-album* from *Gnaphalium* is supported. The species of *Gnaphalium* sect. *Euchiton* are not a coherent group. It appears that *Raoulia* should be split into two genera but with a few species excluded from both segregates. Genus "Z" has close links to neither *Leucogenes* nor *Haastia*. Its separate generic status seems to be justified. The species of *Leucogenes* are closely related. *Haastia* and *Pterygopappus* are not close to each other or to any other genus.

It is shown that leaf anatomical and flavonoid data provide taxonomically useful characters for the classification of the Gnaphaliinae.

CHAPTER ONE

GENERAL INTRODUCTION

This chapter serves as an introduction to the rest of the thesis. It provides an overview of the systematic position and taxonomic problems of the New Zealand Gnaphaliinae as well as the presentation of the aims of this study.

Genera of the Gnaphaliinae in New Zealand and Tasmania

The Gnaphaliinae sensu Merxmüller *et al.* (1977) consists of approximately 95 genera and 1350 species. This subtribe of the Inuleae, Compositae, is distributed world-wide with distinct centres in Australia and South America and less prominent ones in South Africa and the Mediterranean.

This thesis is concerned with the New Zealand and Tasmanian genera of the *Gnaphalium*, *Anaphalis* and *Helichrysum* groups (*sensu* Merxmüller *et al.*, op. cit.) of this subtribe. These groups are represented in the New Zealand flora by seven genera, *Anaphalis* DC., *Ewartia* Beauverd, *Gnaphalium* L., *Helichrysum* L., *Leucogenes* Beauverd, *Pseudognaphalium* Kirpiczn. and *Raoulia* Hook. f.. An eighth genus, Genus "Z", is as yet unpublished (Ward, pers. comm.). The genus *Haastia* Hook. f. (the type species only) may be added here as well (Merxmüller *et al.*, op. cit.). In the Tasmanian flora, *Ewartia, Gnaphalium, Helichrysum, Pseudognaphalium* and *Pterygopappus* Hook. f. belong to these groups.

Anaphalis is represented in New Zealand by four endemic species. These species, included by Allan (1961) in *Gnaphalium*, were recently transferred into *Anaphalis* (Webb, 1987). Drury (1970), describing these species as the anaphalioid group of *Gnaphalium*, suggested this transfer.

Ewartia is a small Australasian genus with one species endemic to New Zealand, three species endemic to Tasmania and a fifth in Victoria and New South Wales.

Gnaphalium is a heterogeneous, cosmopolitan genus. It is subdivisable into four sections, *Gnaphalium, Omalotheca, Gamochaeta* and *Euchiton* (Drury, 1970, 1972). New Zealand has eleven indigenous species of *Gnaphalium* all of which fall within sect. *Euchiton* (Drury, 1972).

Haastia was treated by Allan (op. cit.) as a New Zealand endemic genus of three species in the tribe Astereae. Merxmüller *et al.* (op. cit.) include the type species, *Haastia pulvinaris*, in the Gnaphaliinae and tentatively in the *Gnaphalium* group, suggesting that the two other species "represent quite another genus".

Helichrysum is a large genus of some 500 species distributed throughout Europe, Asia, Africa and Australasia. Bentham, in the *Genera Plantarum* (1873) lists two subgenera of which the first, *Euhelichrysum*, is divided into ten sections. Three of these are represented in the New Zealand flora, of which one, sect. *Leontopodioides*, now has generic status as *Leucogenes*. Of the other two, sect. *Xerochlaena* has two species in New Zealand and sect. *Ozothamnus* has seven; all nine species are endemic.

Leucogenes is a small, New Zealand endemic genus with two species recognised by Allan (op. cit.) and two more yet to be described (Molloy, pers. comm.).

The genus *Pseudognaphalium* was extended by Hilliard and Burtt (1981) to include perhaps 40-50 species including the widespread *Gnaphalium luteo-album* which they place in subgenus *Laphangium*.

Pterygopappus is a monotypic genus endemic to Tasmania.

Raoulia is a New Zealand endemic genus which has long been regarded as heterogeneous (e.g., Hooker 1864, Bentham 1873, Kirk 1899, Allan 1961). According to Ward (1981, 1982), the genus as presently constituted contains two very different species groups, as well as a number of species of uncertain affinities.

Genus "Z", an as yet unpublished genus (Ward, pers. comm.), was described by Allan (op. cit.) under *Leucogenes* "*Incertae Sedis*". It was compared with *Haastia sinclairii* and with *Leucogenes*, and the possibility of a hybrid origin was suggested.

With one exception, no genera other than the ten mentioned above were included in this study.

This exception is the genus *Cassinia* R. Br., also included in the Gnaphaliinae but not belonging to the *Gnaphalium-Anaphalis-Helichrysum* complex. *Cassinia* is represented in New Zealand by one species only, since Webb *et al.* (1988) merged all five species of Allan's *Flora* (op. cit.) into *Cassinia leptophylla*. Tasmania has four species of *Cassinia*.

History and taxonomic problems of the New Zealand Gnaphaliinae

It is apparent from a perusal of the synonymy given in Allan's *Flora* (op. cit.) that there are problems in delimiting these genera. Of the currently accepted species, one in *Gnaphalium* was formerly placed in *Raoulia* (*Gnaphalium mackayi*), two in *Helichrysum* have been referred to *Gnaphalium* (*Helichrysum bellidioides*, and *H. filicaule*), the New Zealand species of *Anaphalis* were known as the anaphalioid group of *Gnaphalium* and both described species of *Leucogenes*, one species of *Raoulia* (*Raoulia youngii*) and one species of *Anaphalis* (*Anaphalis keriensis*) have been placed in both *Gnaphalium* and *Helichrysum*. The single indigenous species of *Ewartia* was earlier placed in *Gnaphalium* and *Helichrysum*. Hybrids between *Raoulia* and *Leucogenes* have been described as species of *Gnaphalium* (*G.* (*H.*) *fasciculatum* Buchan.), *Helichrysum* (*H. pauciflorum* Kirk, *H. loganii* (Buchan.) Kirk, *H. grahamii* Petrie) and *Raoulia* (*R. loganii* (Buchan.) Cheesem., *R. gibbsii* Cheesem.). Of the four Australian species of *Ewartia*, at one time or another two have been placed in *Gnaphalium* and all four in *Raoulia* (Beauverd, 1910, 1911).

Hooker noted in 1864 that *Raoulia* was a genus founded on habit more than on any good characters that could separate it from *Gnaphalium* sect. *Helichrysum*. He considered that the section of *Raoulia* later named sect. *Imbricaria* by Bentham (1873b) probably constituted a good genus, but that the other species (including *R. australis, R. tenuicaulis, R. haastii, R. monroi, R. glabra* and *R. subsericea*) might perhaps fall into *Gnaphalium* or *Helichrysum*. Bentham, in the *Genera Plantarum* (Vol 2, 1873), noted that *Raoulia* is not clearly separable from either *Gnaphalium* or *Helichrysum*, and in the *Flora Australiensis* (Vol 3, 1866) he included *Raoulia* both with *Cassinia* and *Helichrysum* in the subtribe Helichryseae and with *Gnaphalium* and *Antennaria* in the subtribe Eugnaphalieae. Kirk (1899) considered that it was impossible to

maintain *Raoulia* as a separate genus (although he did retain it for convenience) since there were no characters to distinguish it from *Gnaphalium* and *Helichrysum* except the peculiar habit. He suggested placing sect. *Leptopappus* in *Gnaphalium* and sect. *Imbricaria* in *Helichrysum*. Beauverd at first split the genus into two (1910) but later reunited it (1912). Allan (1961) maintained *Raoulia* without comment. The most recent published account of *Raoulia* (Ward, 1982) recognises two very different species groups, as well as a number of species of uncertain affinities, but no formal change has yet been made in the classification.

In the *Handbook of the New Zealand Flora* in 1864, Hooker claimed that the New Zealand species of *Gnaphalium* and *Helichrysum* could not be separated naturally into the two genera. He placed all these species in *Gnaphalium*, but suggested that those with thick pappus hairs broadening at the tip, and with the outer [filiform] florets usually in one series, should perhaps be distinguished from *Gnaphalium* as *Helichrysum* or *Antennaria*. These species were those now known as *Raoulia youngii, Ewartia sinclairii, Leucogenes leontopodium* and *L. grandiceps*; it is interesting to note that the only species which are currently located in *Helichrysum* and which were placed in either genus at the time, namely *H. bellidioides* and *H. filicaule*, Hooker placed without question in *Gnaphalium*! (The other species currently included in *Helichrysum* were placed by Hooker in a separate genus, *Ozothamnus* R. Br.). Relationships of the New Zealand species of *Gnaphalium* have been discussed by Drury (1970, 1972).

The genera *Leucogenes* and *Ewartia* were erected by Beauverd in 1910 and have been accepted by later authors. In this same paper Beauverd considered *Anaphalis*, *Leontopodium* and *Antennaria* in relation to the main genera in this study.

The aim of this thesis

The most extensive study on the New Zealand Gnaphaliinae was undertaken by Ward (1981) who reviewed the classification of *Raoulia* and allied genera in New Zealand with the aid of numerical analysis. Ward argues that the present classification of the Gnaphaliinae is unsatisfactory, because undue reliance has been placed on single characters, such as the floret

ratios in the capitulum of *Gnaphalium* and *Helichrysum*, and because the genera are apparently still in an active state of evolution. A satisfactory classification would not be achieved, according to Ward, until the search for strictly defined genera was replaced by the search for natural aggregations of species.

The confusion in the Gnaphaliinae will best be elucidated by pursuing as many and diverse fields of investigation as possible. The work described in this thesis is part of more extensive research being undertaken on the classification and evolution of the New Zealand Gnaphaliinae, which includes experimental cultivation, hybridisation experiments, cytological investigation, numerical analysis, cladistic analysis and formal taxonomic revision.

The aim of this thesis is to provide evidence for use in the classification and the elucidation of the evolutionary relationships of the species of the Gnaphaliinae by carrying out anatomical and chemotaxonomic studies on the leaves.

Names

The classification system used in this thesis is based on published names. One has to be aware that some species are misplaced, but a start has to be made with taxa in their currently published positions. The names used are mainly those published in *Flora of New Zealand, Volume 4* (Webb *et al.*, 1988). The system of *Flora of New Zealand, Volume 1* (Allan, 1961) was used for species endemic to New Zealand and not commented on by Webb *et al.*. The only exception is *Cassinia*, where Allan's system was applied, since this project started before the five species of *Cassinia* were merged by Webb *et al.*(op. cit.) into one species. *The Student's Flora of Tasmania* (Curtis, 1963) was used for the Tasmanian species.

The species examined in this thesis

Since it would be far beyond the scope of this thesis to examine all species, those worked with were carefully chosen. Representatives of each recognised aggregation and most of the critical species of New Zealand were investigated. Tasmanian genera were examined only

with the purpose of clarifying taxonomic problems of the New Zealand genera. It has to be noted that this study is concerned with problems at the genus level, not at the species level.
The species included in this study are listed in Table 1.1. (Authorities are given in Appendices 1 and 4.)

Anaphalis is represented in this study by five species, including all four species endemic to New Zealand (*Anaphalis rupestris*, *A. keriensis, A. subrigida* and *A. trinervis*) and one species originating in the Himalayas and grown in New Zealand as a garden plant (*A. triplinervis*). *A. triplinervis* was included in this study to investigate the relationship of the New Zealand *Anaphalis* species with a "true" *Anaphalis* species. Webb (1987) transferred the New Zealand species, treated by Drury (1970) as the "anaphalioid group" of *Gnaphalium*, into the quite broadly defined *Anaphalis* and not into the semi-accepted *Anaphalioides* (Benth.) Kirpiczn., on the grounds that the genera are not yet clearly enough defined in this part of the Inuleae for this narrower view to be accepted. It needs to be investigated whether these New Zealand *Anaphalis* species are indeed best placed in *Anaphalis*, whether they should be in *Anaphalioides* or whether they are even a genus on their own.

A second reason for examining all *Anaphalis* species of New Zealand lies within *Helichrysum*. *Helichrysum* is represented in New Zealand, as mentioned above, by sect. *Xerochlaena* and sect. *Ozothamnus*. *Helichrysum* sect. *Xerochlaena* contains two species (*H. bellidioides* and *H. filicaule*). Drury (1971) suggested that *H. bellidioides* belongs with the anaphalioid species of *Gnaphalium*. Webb (op. cit.) regarded a transfer into *Anaphalis* as premature since *H. bellidioides* reputedly hybridises not only with *Anaphalis* but also with species currently treated in *Helichrysum*. Ward's numerical analysis (1981) revealed close links of *Helichrysum bellidioides* with *Anaphalis*. The position of the second species of *Helichrysum* sect. *Xerochlaena* is also uncertain. Ward (1981) argued that the two species of *Helichrysum* sect. *Xerochlaena* might belong to different natural aggregations.

Table 1.1 Species included in this thesis.

Anaphalis		N.Z.	*A. keriensis*
			A. rupestris
			A. subrigida
			A. trinervis
		Himalayas	*A. triplinervis*
Cassinia		N.Z.	*C. fulvida*
			C. leptophylla
		Tasmania	*C. aculeata*
			C. longifolia
Ewartia		Tasmania	*E. catipes*
			E. meredithae
			E. planchonii
		N.Z.	*E. sinclairii*
Gnaphalium		N.Z.	*G. involucratum*
	sect. *Euchiton*		*G. mackayi*
			G. nitidulum
			G. traversii
		Tasmania	*G. umbricola*
Haastia			*H. pulvinaris*
			H. sinclairii
Helichrysum	sect. *Xerochlaena*		*H. bellidioides*
			H. filicaule
			H. coralloides
	sect. *Ozothamnus*	N.Z.	*H. intermedium*
			H. parvifolium
			H. depressum
			H. dimorphum
			H. lanceolatum
		Tasmania	*H. backhousii*
			H. obcordatum
Leucogenes			*L. grandiceps*
			L. leontopodium
		undescribed spp.	*L.* "Marlborough"
			L. "Peel"
Pseudognaphalium			*P. luteoalbum*
Pterygopappus		Tasmania	*P. lawrencii*
Raoulia	subg. *Raoulia*		*R. cinerea*
			R. glabra
			R. hookeri
			R. tenuicaulis
	subg. *Mistura*		*R. petriensis*
	subg. *Psychrophyton*	non-pulvinate species	*R. grandiflora*
			R. hectori
		pulvinate species	*R. bryoides*
			R. eximia
		undescribed spp	*R.* "L"
			R. "M"
undescribed genus			Genus "Z"

Her numerical analysis suggested a possible relationship between *H. filicaule* and *Raoulia cinerea*. One aim of this study was to get more information about the relationships of both species of *Helichrysum* sect. *Xerochlaena*.

Not only the position of the species of *Helichrysum* sect. *Xerochlaena* is questioned, but the position of the New Zealand species of *Helichrysum* sect. *Ozothamnus* as well. Ward (pers. comm.) is separating the species with imbricate leaves from *Helichrysum*. But where does *H. depressum* belong? Does it belong to this new genus or are its affinities with *Raoulia glabra* as tentatively proposed by Ward (pers. comm.)? Another problem is the origin and position of *H. dimorphum*. One hypothesis (Wall, 1919) is that *H. dimorphum* has a hybrid origin with *H. depressum* and *H. filicaule* as the parents. The relationships of *H. lanceolatum* are obscure, although it reputedly hybridises with *H. bellidioides* and possibly with *H. filicaule*. Therefore three species of this new genus in preparation, *H. coralloides, H. intermedium* and *H. parvifolium*, as well as *H. depressum*, *H. dimorphum* and *H. lanceolatum*, were chosen to be included in this study.

As pointed out by Webb *et al.* (1988), the whole group requires further study particularly in relation to the complex in Australia. Therefore two typical members of *Helichrysum* sect. *Ozothamnus* of Tasmania (*H. backhousii* and *H. obcordatum*) were compared with the New Zealand species. The second reason for including these Tasmanian species into the study was that they seem to be very similar to the New Zealand species of *Cassinia*. Hooker (1864) noted that *Ozothamnus* had the characters of *Cassinia*, but without any scales among the florets. He also noted that *C. fulvida* Hook. f., lacking these scales, might be more correctly placed in *Ozothamnus*, and that *C. vauvilliersii* (Homb. et Jacq.) Hook. f. (formerly *O. vauvilliersii*) was scarcely distinguishable from a true *Ozothamnus* of Tasmania, *O. cuneifolius* A.C.. To clarify the position of New Zealand *Cassinia, H. backhousii* and *H. obcordatum* of *Helichrysum* sect. *Ozothamnus*, two species of New Zealand *Cassinia* (*C. fulvida* and *C. leptophylla*) and two species of Tasmanian *Cassinia* (*C. aculeata* and *C. longifolia*) were included.

The three Tasmanian species and the sole New Zealand species of *Ewartia* were studied. One reason for investigating *Ewartia* of New Zealand as well as *Ewartia* of Tasmania is that they are doubtfully congeneric (Ward, 1981). *Ewartia* was erected by Beauverd (1910) to contain the subdioecious species of *Raoulia*. Ward's work suggested that *Ewartia sinclairii* is quite different from the four Australian species. The closest, but still remote, affinity seems to be with *Pseudognaphalium luteoalbum*. More information is necessary to support or refute this hypothesis. A second reason for investigating all Tasmanian species of *Ewartia* is their uncertain taxonomic status. Is the genus *Ewartia*, based only on the subdioecy, really deserving of the status of a genus? Is this group of species congeneric? Which species groups are their closest relatives? One purpose of this study was to provide information for answering these questions.

Ward's (1981) numerical analysis suggested a relationship between the Australian species of *Ewartia* and the gnaphalioid group of *Gnaphalium*. Since *Gnaphalium luteo-album* was transferred into *Pseudognaphalium* (Hilliard and Burtt, op. cit.) and the anaphalioid group of *Gnaphalium* into *Anaphalis* (Webb, 1987), all indigenous New Zealand species of *Gnaphalium* belong to sect. *Euchiton*. Four species were chosen for this study: *G. involucratum, G. mackayi, G. nitidulum* and *G. traversii*. *G. mackayi* is the only New Zealand endemic species included in this study. The only Tasmanian representative is *G. umbricola*. Ward (op. cit.) noted in her numerical analysis that the gnaphalioid group of *Gnaphalium* formed a discrete cluster of uncertain affinities. It was associated with *Raoulia* subg. *Raoulia* as well as with the Australian species of *Ewartia*. Problems involving *Gnaphalium* in this study include the possible affinities of some *Raoulia* species (e.g., *R.* "M") to *Gnaphalium* and the resemblance of *Gnaphalium nitidulum* to *Ewartia planchonii* (Curtis, 1963). Relationships of the New Zealand species of *Gnaphalium* have been discussed by Drury (1970, 1972) and were updated by Webb (op. cit.), but the delimitation and status of *Gnaphalium* within the Gnaphaliinae is still obscure.

Pseudognaphalium luteoalbum was studied for two reasons: firstly, to find out whether the characters used here support Hilliard and Burtt's transfer (op. cit.) of *Gnaphalium luteo-album* out of *Gnaphalium* and if so, how different it is from *Gnaphalium* and whether it is related to any of the groups studied here. Secondly, since Ward (op. cit.) suggested possible affinities to *Ewartia sinclairii*, this hypothesis needs to be tested.

Pterygopappus lawrencii was included in the study because Merxmüller *et al.* (op. cit.) included it within their list of genera for the Gnaphaliinae and suggested a position close to *Haastia pulvinaris*. Merxmüller *et al.* noted that in the Australian region, *Gnaphalium* sect. *Euchiton* seems to be related closely to *Leucogenes*, *Raoulia* and the subdioecious *Ewartia*. He proposed that there might be such relations as well with the perfectly dioecious *Pterygopappus*. Thus, the affinities of *Pterygopappus* were of interest for this study.

Haastia was included for two reasons: firstly, because of the resemblance of *Haastia sinclairii* to the unpublished Genus "Z" (Allan 1961); secondly, because Merxmüller *et al.* (1977) transferred *Haastia pulvinaris* from the Astereae into the Gnaphaliinae and suggested that the remaining two species of *Haastia* represent quite another genus. The two above mentioned species were studied.

The two described and the two unpublished species of *Leucogenes* were examined. The reason for including the genus in this study is its close relationship to other genera of the Gnaphaliinae, as shown for example by its hybridisation with species of *Raoulia* subg. *Psychrophyton*. All four species were studied to provide information for the revision of the *L. leontopodium* group currently being carried out by B.P.J. Molloy.

Genus "Z" was studied to investigate whether the characters used support its generic status. They should provide information about the proposed affinities to *Haastia sinclairii* or *Leucogenes grandiceps*, as well.

Eleven species of *Raoulia* were chosen for this study: five species of *Raoulia* subg. *Raoulia*, the only species of *Raoulia* subg. *Mistura* and five species of *Raoulia* subg. *Psychrophyton*. Each major subgroup of Ward's synopsis (1982) is represented by at least one species.

R. "M" is the only member of species group I-A. *R.* "M" is an unpublished species which forms a rather isolated entity with distant relations to *Raoulia* subg. *Raoulia* and *Gnaphalium* (Ward, 1981).

R. tenuicaulis is in this study the representative of species group I-B. It was chosen since it is regarded as an undoubted member of *Raoulia* subg. *Raoulia*.

Two species of species group I-C were examined. *R. hookeri* of species group I-C-2 represents a second species undoubtedly belonging to *Raoulia* subg. *Raoulia*. *R. glabra* (species group I-C-1) was chosen because of its isolated position within the subgenus and its ability to hybridise with *H. filicaule* and *H. depressum* (Ward, 1981 and pers. comm.).

R. petriensis, forming species group II-A-1, is the only species of *Raoulia* subg. *Mistura*. According to Ward's numerical analysis, *R. petriensis* has no close affinities but is nearest to *Leucogenes*, *Ewartia*, *Raoulia grandiflora* and *R. youngii*. It was hoped by including it in this study to get more information about the relationships of this species.

R. cinerea, species group II-A-2, seems to have its affinities quite outside *Raoulia*. Ward (1981) notes that it appears to be more closely allied to *Helichrysum* sect. *Xerochlaena* than to *Raoulia*.

Species group II-B is represented by *R. hectori*, which has links to both pulvinate and non-pulvinate species of *Raoulia* subg. *Psychrophyton* (Ward, 1981).

R. grandiflora represents species group II-C. An alliance of *R. grandiflora* with *Leucogenes* has been suggested (Ward, 1981), but more information is needed about its affinities.

Species group III contains the tap-rooted cushion plants of *Raoulia*. *R. eximia* represents species group III-A. Two species of species group III-B were examined: *R. bryoides* and *R.* "L".

Leaf anatomical and chemotaxonomic studies on the *Raoulia* group were undertaken with the aims of firstly elucidating the relationships of the well-defined species groups within the Gnaphaliinae and secondly obtaining more information about the affinities of those species of uncertain status.

In summary, the subtribe Gnaphaliinae is characterised by genera which are poorly delimited morphologically and which, in New Zealand at least, are not yet genetically isolated. Therefore, to increase the knowledge about this most complicated group, this thesis presents a study of the leaf anatomy and chemotaxonomy of the New Zealand and Tasmanian Gnaphaliinae.

CHAPTER TWO

LEAF ANATOMY

2.1 Introduction

Carlquist (1961) has stated that "certainly no generic monograph can be said to be complete without studies on leaf anatomy." The leaf is anatomically very variable and this provides many features of potential taxonomic significance.

Since Radlkofer's (1875) early taxonomic work on the anatomy of *Serjania*, numerous works on comparative anatomy of leaves have been published, e.g., Edelhoff, 1886; Bailey *et al.*, 1944; Abu-Asab *et al.*, 1987; Keating, 1982. Although comparative anatomy was most popular in the last decades of the last century and the early ones of this century it is still considered to be of great value today (Carlquist, op. cit.).

There was and is a lot of interest in the anatomy of the Inuleae (Himmelbauer and Federanko, 1933; Gattiker 1939; Freire, 1986). Even the leaf anatomy of some New Zealand species of the Gnaphaliinae has been investigated. (Remarkable specialisations of habit and habitat make them conspicuous targets for anatomical studies.) Lazniewski (1896) published a short account of the leaf anatomy of *Ozothamnus selago* Hook. f.(now included in *Helichrysum intermedium* Simpson) and *Haastia pulvinaris*, while Low (1899) undertook more detailed studies on the latter. Diels (1896) studied the leaf anatomy of *Ozothamnus coralloides* Hook. f. [= *Helichrysum coralloides* (Hook. f.) Benth. et Hook. f.]. Foweraker (1917) included leaf anatomical studies of *Raoulia tenuicaulis, R. glabra, R. australis* Hook. f. (= *R. hookeri* Allan), *R. subsericea, R. monroi, R. lutescens* Beauverd and *R. haastii* in his investigations of the mat- and cushion-plants of the Cass river-bed. The leaf anatomy of *Gnaphalium traversii*, *Helichrysum bellidioides* and *Cassinia vauvilliersii* was described by Betts (1920a, 1920b). Hauri (1915) undertook anatomical studies on cushion-plants, including *Pterygopappus lawrencii* and several species of *Raoulia.*

These anatomical studies, however, were not taxonomically oriented. In this study now presented, a detailed comparative anatomical survey was undertaken. The information obtained should aid in clarifying the systematic relationships of the New Zealand Gnaphaliinae and their Tasmanian closest relatives. It is hoped to demonstrate this in the following account.

2.2. Materials and methods

Materials

Fresh plant material of almost all taxa was collected on field trips in the South Island (New Zealand) in 1987, in Tasmania in 1988 and in the North Island (New Zealand) in 1989. *Leucogenes* "Marlborough" and *Leucogenes* "Peel" were obtained from the experimental gardens of Botany Division, D.S.I.R, at Lincoln. *Anaphalis triplinervis* was grown in the glasshouses of the Department of Plant and Microbial Sciences, University of Canterbury, New Zealand.

All plant material was collected between January and the end of March. Only mature, healthy leaves getting a lot of light exposure were used for analyses. Two collections of each species were examined. Herbarium specimens of all plants examined are deposited at CANU (= University of Canterbury Herbarium). Collecting data are given in Appendix 1. Permanent slides of the leaf sections are deposited at the Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand.

Methods

Histological procedures for light microscopy

Resin embedding

The method applied in the processing of the plant material followed partly standard procedures (O'Brien *et al.*, 1981; Polysciences, Inc., 1987), partly procedures routinely used in the electron microscopy laboratory of the Department of Plant and Microbial Sciences, University of Canterbury.

Pieces the full width of each leaf and a few millimetres in length were excised from halfway between the base and the apex of the lamina of fresh leaves. These were fixed for 24 hours under vacuum in buffered (0.025M Sörenson's phosphate buffer, pH 7.2) 3% glutaraldehyde (50% biological grade) and then passed through an ethanol series with 20 minute changes each in 20, 40, 60, 80 and 3x100 per cent ethanol before they were infiltrated in solution A (100 ml) and the accelerator benzoyl peroxide (0.9 g) of the JB-4 embedding kit (Polysciences, Inc. embedding kit Cat. # 00226). Infiltration in this catalysed solution A was continued for three weeks at 4°C, then the specimens were embedded in JB-4 (one part by volume of solution B was added to 25 parts by volume of catalysed solution A with stirring) in polythene capsules. Since oxygen inhibits polymerisation the polythene capsules were kept for several hours under a bell filled with nitrogen . The embedded leaves were sectioned on a Jung rotary microtome equipped with glass knifes made on a LKB 2078 Histo knife maker. Transsections of 4 μm thickness were stained in aq. Methylene blue/Azure blue. Permanent slides were prepared by mounting in a xylene based mounting medium (Depex).

Modifications to these procedures

It was very difficult to obtain satisfactory embedding of most of the tomentose leaves. Several modifications of the above procedures were tried. For example, ethanol was added to the embedding solution, the leaves were evacuated at each step of dehydration, 1% acrolein and/or either 1 drop of detergent or ethanol was added to the fixative, the leaves were shaken in chloroform prior to fixation. Removing the tomentum with a sharp scalpel was also tried. Best

results were obtained by adding a drop of detergent to the embedding solution to reduce the surface tension during embedding. Not all specimens were perfectly embedded, but satisfactory leaf sections could be obtained since all specimens were fixed and infiltrated well. Obviously different histological procedures are required to produce optimum results in different leaves. Since this is a comparative study, however, it was considered more important to treat all specimens in a similar manner.

Additional histological methods

Hand sectioning

Plant material was preserved in FAA (Formalin-acetic-alcohol). Specimens were excised and handcut with razor blades (GEM). Sections were examined under the light microscope as aqueous mounts.

Paraffin embedding

For paraffin embedding the method of Johansen (1940) was followed. Specimens were fixed in FAA and dehydrated in a graded TBA (2 methyl propan-2-ol) series. They were infiltrated in 50/50 TBA/liquid paraffin and embedded in Gurr's paraffin wax. Sectioning at 10 μm was followed by staining with Safranin and Fast Green.

Light microscopy

Sections were routinely examined using a Leitz diaplan comparison microscope equipped with a Wild photographic unit. All leaf anatomy descriptions were prepared from observations of transverse sections made perpendicular to the midrib and margin with the adaxial surface orientated uppermost in description, figure and plate orientation.

Procedures for scanning electron microscopy

Leaf material was prepared following the freeze-fracture/freeze-drying procedures of Fineran and Condon (1988). The leaves were frozen initially in the form of "slush", that is, sub-cooled liquid nitrogen (Robards and Sleytr, 1985). The slush was prepared by evacuating liquid nitrogen, within a polystyrene container, in a vacuum evaporator (Edwards model) until solid nitrogen was produced. At atmospheric pressure, slush quickly formed and the specimen was immediately plunged into it. Specimens were fractured while immersed in the cryogen by gripping the leaf with two pairs of forceps and "bending" it until it snapped. After freezing and fracturing the leaves were freeze-dried using a vacuum evaporator and a Bullivant-Ames freeze-fracture device (Bullivant, 1969), minus its lid. Freeze-drying took place over 11 hours, under a vacuum pressure of approximately 10^{-1} Torr maintained by a rotary pump. The freeze-dried specimens were mounted on aluminium stubs, held with copper conductive adhesive (copper print, G.C. Electronics, Illinois U.S.A.) and coated with gold/palladium in a Polaron E 5000 sputter unit. A Cambridge Stereoscan 250 MK II scanning electron microscope was used for examination of the samples.

Photography

Ilford FP4 35 mm films were used. The photographic paper was Ilford 2 grade. All photographs in this thesis are calibrated in microns by means of bar scales. Bar scales correspond to 50 micron increments.

Numerical analyses

The basic data matrix was formed by 49 taxa (OTUs) of the Gnaphaliinae and 49 characters. Similarities between the taxa were calculated using Gower's general coefficient of similarity. The similarity values were clustered by the unweighted pair group method using arithmetic averages (UPGMA) and by the single linkage technique. The degree of fit of a phenogram to the similarity matrix from which it is derived was measured using the cophenetic correlation coefficient of Sokal and Rohlf (1962).

The program used for the numerical analyses was "Gower", written by Drs. C.M. Frampton, G.A. Findlay and J.M. Ward, Christchurch.

Cladistic analysis

A data matrix of 25 characters and 37 evolutionary units (EUs) was used. This was derived from the basic data matrix of 49 characters and 49 species. Quantitative (measurement) and ratio characters were excluded, following the recommendations of Pimentel and Riggins (1987). Species having identical states or expressions of all remaining characters were combined into a evolutionary unit (EU). Character states being found in only one EU were then excluded.

The data matrix was analysed using Swofford's (1985) package PAUP with the Tasmanian species of *Cassinia* as the out-group and with the options ADDSEQ=CLOSEST (determining the order of taxon addition during tree construction), OPT=FARRIS (specifies algorithm) and HOLD=5 (number of trees retained in memory at each step in tree construction). Global branch swapping with MULPARS was performed in a search for multiple, equally parsimonious solutions. The limit of 100 trees was specified by MAXTREE. A strict consensus tree (Rohlf, 1982) for equally parsimonious trees found in the analysis of the data matrix was obtained by running CONTREE.

2.3. Terminology

2.3.1. Numerical analysis

Characters

The characters were coded in the data matrix as "*continuous*", "*discrete non-ordered*" or "*binary dichotomous*".

Characters designated "*continuous*" in the computer program are *ordered multi-state characters*. These characters, also called *quantitative multi-state characters*, are those in which the character states can be placed in an ordered sequence. Such characters may have a finite number of states or they may be a series of measurements along a scale. In the latter case they are sometimes called *continuous characters*.

Characters designated "*discrete non-ordered*" in the computer program include *non-ordered multi-state characters* (in which the character states cannot be placed in an ordered sequence) and *binary alternative characters* (in which shared negative matches are counted as similarities).

Binary dichotomous characters are those in which shared negative states are not counted as similarities; they are ignored.

Operational taxonomic units

The taxa used in a numerical analysis are referred to as operational taxonomic units (OTUs). In this study the OTUs are species.

Similarity coefficients

Gower's general coefficient of similarity (1971) can be used with data containing different kinds of characters without the necessity for recoding. Gower's coefficient is a composite of three different similarity coefficients. One of the three is chosen for each character in the data set. Jaccard's coefficient S_J is used with binary dichotomous characters (shared absence or negative state of the character not scored as a similarity), the simple matching coefficient S_{SM} is used with "discrete non-ordered" characters (sharing of any or either state of the character scored as a similarity). With "continuous" characters Gower applies the following coefficient:

$$S_{ijk} = 1 - (| X_{ik} - X_{jk} | / R_k)$$

where X_{ik} is the score of OTU **i** for character *k*, X_{jk} is the score of OTU **j** for character *k* and R_k is the range of character *k*.

The simple matching coefficient of Sokal and Michener (1958) is defined as:

$$S_{SM} = (Nsp + Nsn)/(Nsp + Nsn + Nu)$$

and Jaccard's coefficient (1908) is defined as:

$$S_J = Nsp/(Nsp + Nu)$$

where Nsp is the number of states whose presence or positive state is shared by two OTUs, Nsn is the number of shared absence or negative states in the two OTUs being compared and Nu is the number of unshared states (i.e., present/positive in one and absent/negative in the other of the two OTUs being compared). The simple matching coefficient gives equal weight to the shared presence/positive state and absence/negative state of characters, while Jaccard's coefficient ignores shared absences/negative states.

Cluster methods

The similarity values were clustered by the unweighted pair group method using arithmetic averages (UPGMA) and by the single linkage technique.

In UPGMA, an OTU has a similarity to an existing cluster equal to its average similarity to the members of the cluster (Sokal and Michener, 1958). The similarity between two clusters is equal to the average similarity of all members of one cluster with all members of the other. UPGMA provides information on average phenetic relationships. The clusters form over an intermediate range (compared with single and complete linkage clustering) and the hierarchical

structure is quite clear. UPGMA generally gives the least amount of distortion of a similarity matrix (Rohlf, 1970; Sneath and Sokal,1973). However, outlying OTUs (those which are not similar to any others) may form a pair not because they are most similar to each other, but rather because their similarity to each other is higher than either one's average similarity to any existing cluster.

In single linkage clustering, an OTU has a similarity to an existing cluster which is equal to its similarity to the closest member within the cluster. The single linkage technique (Florek *et al.*, 1951a,b; Sneath, 1957) provides information on closest phenetic relationships, and because of the criterion for entry into and fusion of clusters it is not sensitive to cluster size.

Cophenetic correlation coefficient

Since the phenogram used to show the results of cluster analysis is a two-dimensional representation of a multi-dimensional structure, some distortion of the relationships in the similarity matrix on which it is based is inevitable. The degree of fit of a phenogram to the similarity matrix from which it is derived may be measured using the cophenetic correlation coefficient proposed by Sokal and Rohlf (1962). A matrix of cophenetic values is obtained from the phenogram by finding the similarity level that links each pair of OTUs. The cross-product correlation coefficient is then computed between the two matrices; this is the cophenetic correlation coefficient. A value of one represents complete agreement between the two matrices.

2.3.2. Cladistic analysis

The groups of study organisms in a cladistic analysis are referred to as evolutionary units (EUs).

A *cladistic analysis* is a method that attempts to recover genealogical relationships among groups of organisms. These relationships are often displayed as a branching diagram known as a *cladogram*.

The cladistic approach to character analysis has resulted in the recognition that the only groups worthy of discussion are *monophyletic* groups. A monophyletic group is one that includes the common ancestral species plus all of the descendant species. *Sister groups* are two taxa which are considered to have a common ancestor not shared with any other taxon.

The number of possible character state changes provides the *range* of a character. The range of a character is equal to the number of character states minus 1. For any given tree, the number of character state changes per character is known as the *length* of the character. The ratio of range to length is known as the *character consistency index*.

Characters having a common origin are *homologous*. A character derived from its pre-existing homologue is termed *apomorphic*. An attribute unique to one evolutionary unit and thought to originate in that evolutionary unit is termed *autapomorphic*. A unique character among a group of individuals which is found in their common ancestor and thought to have originated in that ancestor is termed *synapomorphic*. The original pre-existing character from which its homologous character was derived is termed *plesiomorphic*.

The operational method used in this thesis for assessing polarity among character states is *out-group comparison*. An *out-group* is a group of organisms that is related to but removed from the group of the study taxa. One or more out-groups are examined to determine which character-states are evolutionary novelties.

A number of phylogenetic methods have been developed, the most popular of which is *parsimony analysis*. Parsimony attempts to find the cladogram postulating the least number of character-state changes. The most parsimonious tree is taken as a hypothesis of evolutionary history.

2.4. Results

2.4.1. Nature and distribution of anatomical characters

Lamina thickness: The thickness of the lamina, measured halfway between margin and midvein, ranges from 100 to 400 μm. The species of *Anaphalis* have the thickest leaves and *Helichrysum filicaule* the thinnest leaves.

Lamina structure: The lamina structure in the Gnaphaliinae is diverse. Flach's table (1916, ex Napp-Zinn 1974) of types of lamina structures was a useful guide for tabulating different types. The terms of Napp-Zinn (1974) are used for the lamina structure. All leaves in this study are bifacial leaves. Bifacial leaves are defined as having an upper and a lower side, in contrast to unifacial leaves in which one of these sides is absent. Napp-Zinn prefers to use the terms "Oberseite" and "Unterseite" instead of "adaxial" and "abaxial", since the latter terms are often correctly applied only for leaves still in the stage of leaf buds. However, bearing this in mind, for ease of use the terms "adaxial" and "abaxial" are used in this thesis. Dorsiventral leaves are leaves in which the mesophyll consists of adaxial palisade parenchyma and abaxial spongy parenchyma with the stomata usually mainly or solely on the abaxial surface. Inverse-dorsiventral leaves are leaves in which the palisade parenchyma is on the abaxial side and the spongy parenchyma on the adaxial side with the stomata mainly or solely on the adaxial surface. Equifacial leaves are leaves in which the epidermis and assimilation tissues of the abaxial and adaxial sides of bifacial leaves are symmetrical and the stomata are mostly equally common on both sides. The mesophyll may have cells all of the same kind, when it is called homogeneous mesophyll, or it may have palisade parenchyma on both sides with a different tissue in the middle. The species examined show many different bifacial transitional leaf types from equifacial to dorsiventral (Table 2.1; Figure 2.1).

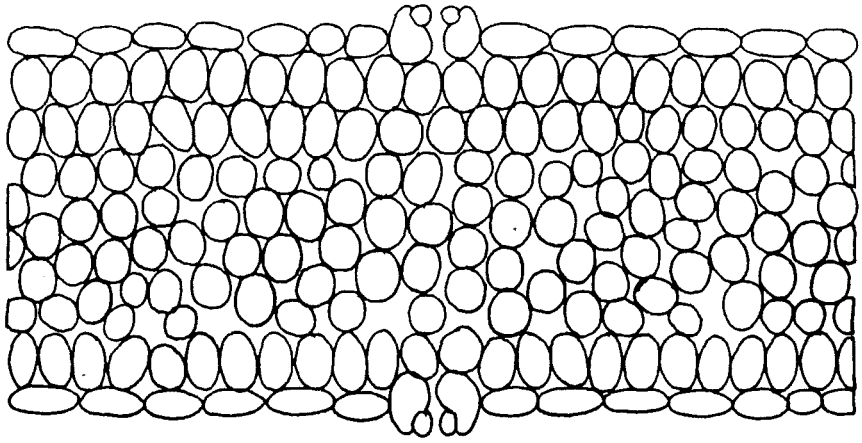
Table 2.1. Lamina anatomy types.

Bifacial

1. equifacial or almost equifacial, stomata equally on both sides
    - 1.1. mesophyll poorly differentiated: oval/round/oval cells
        - *E. catipes, R. bryoides, R. eximia, R. hectori, R.* "L"
    - 1.2. mesophyll with oval cells on both sides, middle cells large
        - *R. glabra, R. hookeri, R. tenuicaulis*
    - 1.3. mesophyll with equal palisade parenchyma on both sides, middle cells medium-sized
        - Genus "Z"
2. almost equifacial, stomata ad
    - 2.1. mesophyll almost homogeneous
        - *Pterygopappus lawrencii*
    - 2.2. mesophyll poorly differentiated: ad oval/ab round cells
        - *R. grandiflora*
3. transitional, closer to equifacial, mesophyll with palisade parenchyma ad and round middle cells, stomata ad to equal on both sides
    - 3.1. oval cells ab and small middle cells, stomata more ad
        - *R. cinerea*
    - 3.2. round cells ab and large middle cells
        - *L. grandiceps* (stomata ad > ab), *L. leontopodium* (stomata ad > ab), *L.* "Peel" (stomata ad = ab), *L.* "Marlborough" (stomata ad)
4. transitional, closer to dorsiventral, mesophyll with palisade parenchyma ad and small middle cells, stomata more ab than ad
    - 4.1. mesophyll with ab cells palisade-like, shorter than ad
        - *G. nitidulum*
    - 4.2. mesophyll with ab cells round or horizontally elongated
        - *E. sinclairii, G. mackayi, G. traversii*
5. dorsiventral, mesophyll with palisade parenchyma ad, spongy parenchyma ab
    - 5.1. stomata ab
        - 5.1.1. palisade cells, spongy parenchyma uniform
            - *Anaphalis, Cassinia, E. meredithae, G. involucratum, G. umbricola, (Haastia), H. backhousii, H. bellidioides, H. dimorphum* (normal leaf), *H. obcordatum*
        - 5.1.2. palisade cells, spongy parenchyma cells larger towards ad
            - *H. lanceolatum*
        - 5.1.3. palisade-like cells, spongy parenchyma uniform
            - *E. planchonii*
        - 5.1.4. mesophyll of irregularly shaped cells, more closely packed and vertically oriented ad, horizontally ab
            - *Raoulia* "M"
    - 5.2. stomata more ab than ad
        - *H. filicaule*
    - 5.3. stomata equally on both sides
        - *Pseudognaphalium luteoalbum*
6. inverse-dorsiventral, mesophyll with palisade parenchyma ab, spongy parenchyma ad, stomata ad
    - 6.1. palisade cells
        - *H. coralloides, H. depressum, H. dimorphum* (scale-like leaf), *H. intermedium, H. parvifolium*
    - 6.2. palisade-like cells
        - *R. petriensis*

Key: ad = adaxial; ab = abaxial.

1.1.



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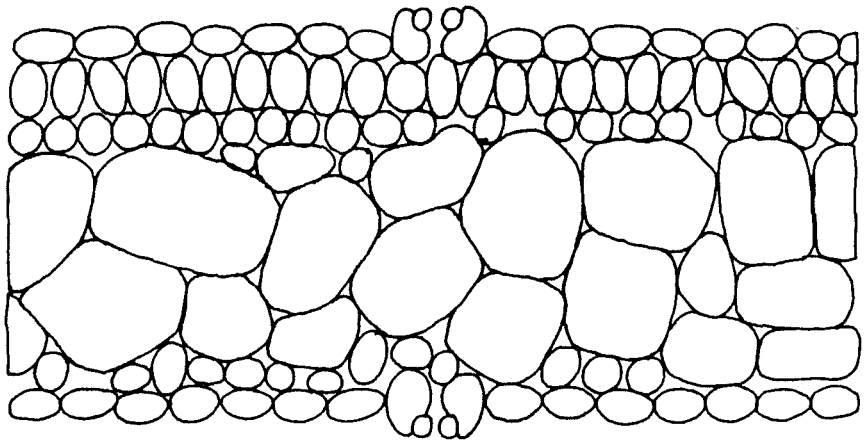

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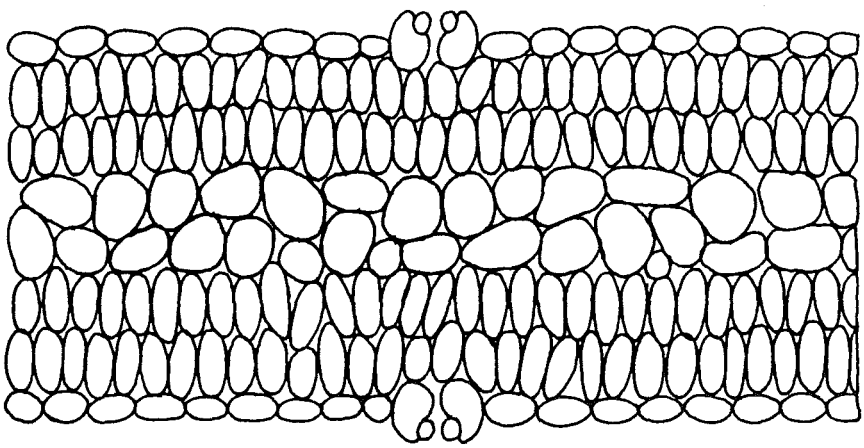

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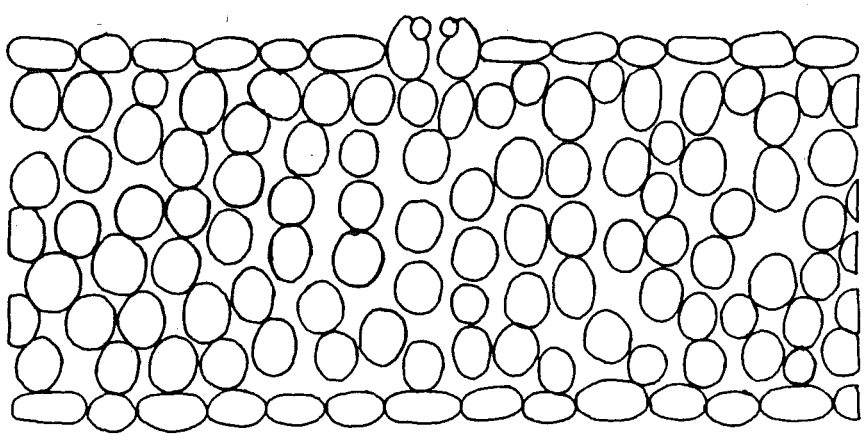
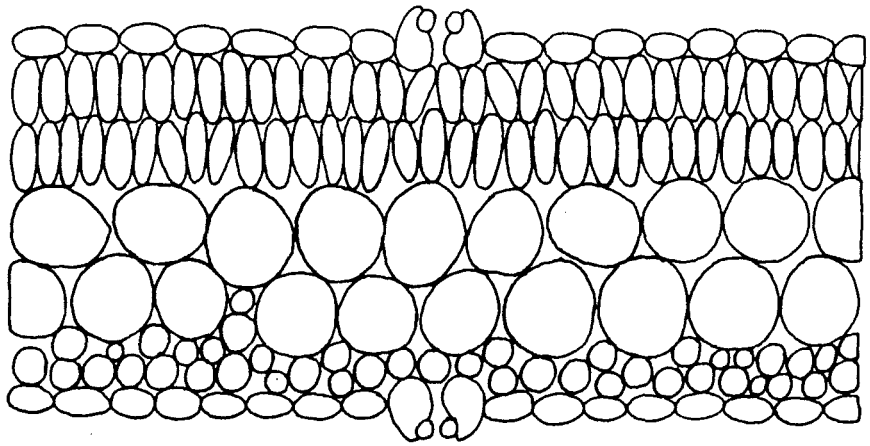


Figure 2.1. Illustrations of selected lamina anatomy types from Table 2.1.

3.2.



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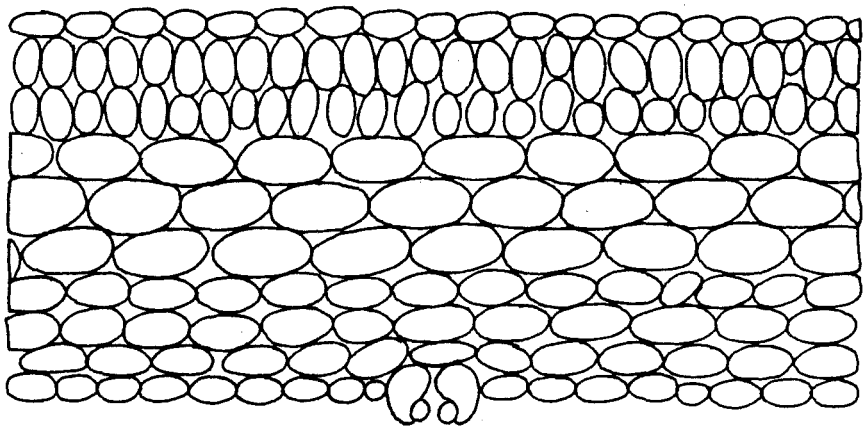
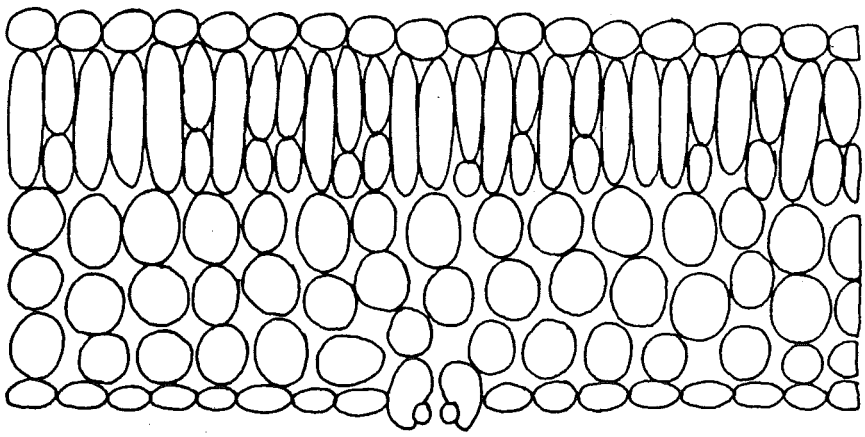


5.1.1.



5.1.3.

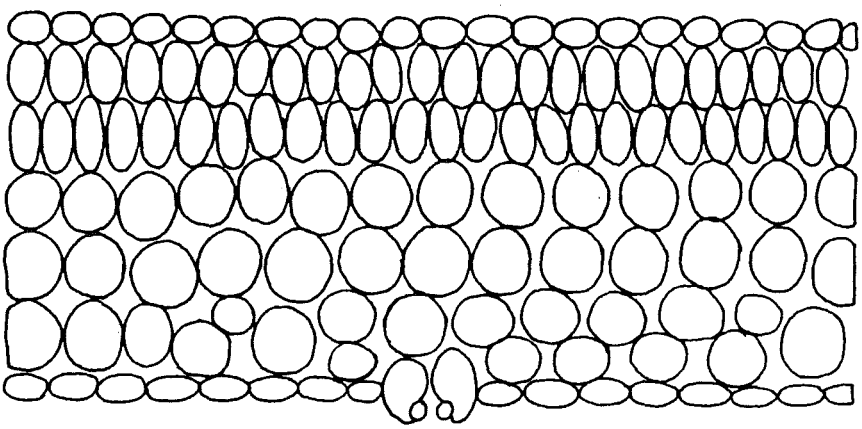


Figure 2.1. continued.

Cuticle: The cuticle varies from less than 5 μm in, for example, *Raoulia tenuicaulis* (Plate 17, A) to 40 μm in *Helichrysum intermedium*. It may be of equal thickness on both surfaces, thicker on the abaxial or adaxial surface or sometimes thickened at the margin (e.g., *Leucogenes leontopodium* Plate 12, E) or the midrib (e.g., *Gnaphalium involucratum* Plate 6, E and F). In most instances the cuticle is of even thickness on homogeneous or equifacial leaves, thicker on the adaxial side of dorsiventral leaves and thicker on the abaxial side of inverse-dorsiventral leaves. Exceptions include *Pterygopappus lawrencii* (Plate 14, A and B), in which the cuticle is thicker on the abaxial side of the homogeneous leaves and *Cassinia aculeata* (Plate 2, E; Plate 3, B), in which it is of even thickness on both sides of the dorsiventral leaves. If the cuticle is conspicuous, it is flanged between the epidermal anticlinal walls and sometimes reaches the inner periclinal wall of the epidermis (e.g., *Anaphalis keriensis*, Plate 1, A). In such cases the boundary between the cuticle and the epidermal wall could not be discerned. Therefore the thickness of the epidermal wall was included in the cuticle thickness measurement.

Epidermis: The epidermis normally consists of one layer of isodiametric to long-oval cells. The abaxial epidermal cells of the inverse-dorsiventral species of *Helichrysum* (Plate 10, A, E, F), however, are almost rectangular and often higher than wide, while the epidermis of *Pterygopappus lawrencii* is in places two cells deep, in which case the cells are much smaller than the normal epidermal cells (Plate 14, A and B).

The shape of the epidermal cells is usually fairly regular. *Pseudognaphalium luteoalbum*, however, has irregularly shaped epidermal cells on both sides (Plate 13, E and F), and irregularly shaped abaxial cells are common in *Gnaphalium involucratum*, *G. umbricola*, *Anaphalis triplinervis*, *Helichrysum backhousii* and the New Zealand *Cassinia* species (e.g., *C. leptophylla*, Plate 3, E).

The height of the epidermal cells ranges from 10-50 μm. The height of the adaxial and abaxial epidermal cells is either similar or dissimilar. Most dorsiventral leaves have taller epidermal cells on the adaxial side (e.g., *Anaphalis keriensis*, Plate 1, A), while inverse-dorsiventral leaves have taller cells on the abaxial side (e.g., *Helichrysum parvifolium*, Plate 10, A). The dorsiventral leaves of the Tasmanian *Cassinia* species, however, have epidermal cells of equal height (e.g., *C. aculeata*, Plate 3, B). The epidermal cells of equifacial leaves are more or

less equal in height on both sides (e.g., *Raoulia tenuicaulis*, Plate 17, A). The abaxial epidermal cells of dorsiventral leaves may be higher in the midrib (e.g., *Gnaphalium involucratum,* Plate 6, E; *Anaphalis triplinervis,* Plate 2, C).

Modified cells in the adaxial epidermis of the midrib are found *Pterygopappus lawrencii* and all species of *Anaphalis*. *Pterygopappus lawrencii* has one big cell of 35 μm in diameter, while the other epidermis cells are less than half this size (Plate 14, A). All species of *Anaphalis* have four to eight epidermal cells which are much narrower than the other cells (e.g., *A. subrigida*, Plate 1, D; *A. triplinervis*, Plate 2, C).

Stomata: All specimens examined have small guard cells of 5-10 μm in diameter and subsidiary cells which are not differentiated from other epidermal cells. The subsidiary cells are approximately twice the size of the guard cells. The cuticle at the outer guard cell wall is thick and outer stomatal ledges may occur here (e.g., *Anaphalis triplinervis*, Plate 2, D).

Stomata may occur on one surface only, or on both surfaces in equal or in unequal numbers. Dorsiventral leaves usually have stomata confined to the abaxial surface (e.g., *Anaphalis keriensis*, Plate 1, A), while inverse-dorsiventral leaves have the stomata on the adaxial surface only (e.g., *Helichrysum depressum*, Plate 10, E and F). However, *Pseudognaphalium luteoalbum*, with dorsiventral leaves, has stomata in equal numbers on both surfaces and *Helichrysum filicaule*, also with dorsiventral leaves, has most of the stomata on the abaxial, but also some on the adaxial, surface. There are many forms of equifacial leaves and transitional forms between equifacial and dorsiventral leaves and they show different stomata distributions as well. The equifacial leaf of *Raoulia grandiflora* (Plate 16, A and B), for example, has stomata only on the adaxial surface, Genus "Z" (Plate 18, D) with equifacial leaves has equal numbers of stomata on both surfaces and *Gnaphalium mackayi* (Plate 7, A) has more stomata on the abaxial surface. The species of *Haastia* (e.g., *H. sinclairii*, Plate 8, F) have stomata on the abaxial surface at the middle of the leaf blade, but on both surfaces further towards the leaf apex.

The stomata can be level with the unspecialised epidermal cells (e.g., Genus "Z", Plate 18, E), slightly raised, i.e., only the guard cells are raised (e.g., *Anaphalis keriensis*, Plate 1, A), raised, i.e., the subsidiary cells are raised as well (e.g., *Cassinia fulvida*, Plate 3, F), or extremely

raised, i.e., the subsidiary cells and often the cells adjacent to the subsidiary cells are very much raised (e.g., *Cassinia aculeata*, Plate 2, E).

Substomatal chambers vary from small to medium to large. Small substomatal chambers are defined as normally reaching less than half the leaf width (e.g., *Leucogenes leontopodium*, Plate 12, E), medium ones as reaching half the leaf width (e.g., Genus "Z", Plate 18, E) and large ones as reaching more than half the leaf width (e.g., *Anaphalis keriensis*, Plate 1, A).

Mesophyll: There are two types of palisade cells, namely normal rod-shaped palisade cells (e.g., *Helichrysum obcordatum*, Plate 12, A) and oval to round palisade cells, here called "palisade-like cells"(e.g., *Ewartia planchonii*, Plate 5, C; *Raoulia bryoides*, Plate 14, C and D). The cells are regarded as palisade cells if they are on average more than twice as long as wide and as palisade-like cells if they are less than twice as long as wide, except that all short cells (less than 30 μm) are treated as palisade-like cells. The length/width ratio of the palisade and palisade-like cells ranges from 4.0 in *Helichrysum obcordatum* (Plate 12, A) to 1.2 in *Ewartia catipes* (Plate, 4, A, C and D). The length of palisade and palisade-like cells ranges from 15 μm (e.g., *Raoulia petriensis*, Plate 18, B) to 70 μm (e.g., *Ewartia meredithae*, Plate 5, A). The maximum number of palisade rows is found in *Cassinia fulvida* (Plate 3, F) and *Helichrysum backhousii*, both of which can have up to six rows of palisade cells. Most species, however, have 1-2 rows. The measurements of the palisade cells are taken from cells adjacent to the epidermis. Palisade cells in the second and following rows are generally smaller.

Spongy parenchyma is found only in dorsiventral leaves. The cells of the spongy parenchma are usually elongated parallel to the leaf surface or less commonly are isodiametric. Sometimes it is quite difficult to distinguish them from palisade-like cells, for example in *Raoulia* "M" (Plate 17, C, E and F). The length of the spongy parenchyma cells ranges from 15 μm in *Raoulia* "M" up to 80 μm in the species of *Anaphalis* (e.g., *A. keriensis*, Plate 1, A). The ratio of palisade/spongy parenchyma ranges from 0.3 in *Anaphalis keriensis* (Plate 1, A) to 2.2 in *Haastia sinclairii* (Plate 8, F).

Many leaves have a central tissue of polygonal or roundish to elongated, clear cells. This central tissue is of three types. In the first, the cells are polygonal, on average 60 μm long

(with individual cells up to 80 μm long), occur in one to three layers and occupy much of the thickness of the lamina. These large middle cells are found in *Raoulia glabra, R. hookeri* and *R. tenuicaulis* (e.g., Plate 17, A, *R. tenuicaulis*). In the second type of central tissue the cells are medium-sized, usually round, and less conspicuous. These cells are on average 40 μm long (with individual cells up to 60 μm long) and occur in the species of *Leucogenes* (e.g., *Leucogenes* "Peel", Plate 13, A). The cells of *Leucogenes grandiceps*, however, differ from those of the other three species in shape, being polygonal rather than round. The cells of the third type are on average 25 μm long (with individual cells up to 30 μm long) and horizontally elongated. These small middle cells are found in Genus "Z", *Raoulia cinerea, Ewartia sinclairii, Gnaphalium mackayi, G. nitidulum* and *G. traversii* (e.g., *G. mackayi*, Plate 7, C).

Margin: In most species the leaf margin is rounded, but it is pointed in *Anaphalis subrigida* (Plate 1, E) and *A. trinervis*. The palisade cells at the margin are either restricted to one of the surfaces (e.g., *Gnaphalium involucratum*, Plate 6, C) or continuous around the periphery of the leaf (e.g., *Raoulia hookeri*, Plate 17, D) or are missing from the the marginal area (e.g., *Helichrysum parvifolium,* Plate 10, B).

Midvein: All species have a collateral midvein, the diameter of which ranges from 25-35 μm in *Pterygopappus lawrencii* to 200-220 μm in *Cassinia longifolia*.

Sclerenchyma caps may occur on either one or both sides of the midvein with the position being constant in any given species. (It is emphasised that cross-sections taken halfway between the lamina base and apex were compared and the amounts of sclerenchyma may be different in other parts of the leaf). The cell walls are so thick that there is no or almost no lumen left. Sclerenchyma caps were observed on the abaxial side of the midvein in *Helichrysum coralloides, H. intermedium* (Plate 10, C), *H. parvifolium* and *Raoulia bryoides*. Sclerenchyma caps occur on the adaxial side of the midvein in *Raoulia eximia, R. hectori* and *R. grandiflora* (Plate 15, E), and on both sides of the midvein in all *Leucogenes* species (e.g., *L. leontopodium*, Plate 12, F), except *L. grandiceps* (Plate 12, D), which has no sclerenchyma caps at all.

The midveins of some species have caps of thick-walled cells (here distinguished as "thick-walled cells") which have much more lumen than those of the sclerenchyma caps mentioned above. *Anaphalis rupestris*, for example, has caps of thick-walled cells on the adaxial side (Plate 1, C) and *Ewartia meredithae* (Plate 4, E) has them on both sides.

Midrib: The profile of the adaxial and abaxial surfaces varies among the species. There are all transitions from an abaxially very prominent midrib to a totally immersed midrib. The amount of protrusion was expressed by measuring the vertical distance in μm from the abaxial surface of the lamina to the apex of the midrib. *Gnaphalium involucratum* (Plate 6, E), for example, has leaves with very protruding midribs. The midrib of *Cassinia aculeata* is acute (Plate 2, E). Less protruding midribs are found, for example, in *Gnaphalium mackayi* (Plate 7, A), while the midribs of others, including *Leucogenes* (e.g., *L. grandiceps*, Plate 12, D) are not protruding at all. No species have the midrib protruding on the adaxial side; it is slightly indented in *Cassinia longifolia* and the New Zealand species of *Anaphalis* (e.g., *A. subrigida*, Plate 1, D) and very much indented in *Cassinia aculeata* (Plate 2, E). The palisade mesophyll may be continuous across the midrib (e.g., *Cassinia aculeata*, Plate 2, E) or it may be replaced by collenchyma (e.g., *Gnaphalium involucratum*, Plate 6, E and F). Many species have collenchyma cells surrounding the midvein. The collenchyma cells may fill the whole remaining midrib, as in *Gnaphalium involucratum* (Plate 6, E), or be confined to the abaxial side of the midvein, as in *Cassinia leptophylla* (Plate 3, C), or surround the vein but not reach the epidermis as in *Helichrysum filicaule* (Plate 11, E). Some cells on the abaxial side of the midvein have thickened walls in Genus "Z" (Plate 18, C).

The midvein is, except in *Helichrysum lanceolatum*, always surrounded by a parenchymatous bundle-sheath, which in midribs filled with collenchyma cells is often not very obvious, but otherwise is quite conspicuous. The bundle-sheath is generally single-layered. The New Zealand *Anaphalis* species, however, have a bundle-sheath of several layers with cells smaller than the surrounding collenchyma cells (e.g., *A. rupestris*, Plate 1, C). The bundle-sheath cells of *Helichrysum lanceolatum* have thickened walls.

Lateral ribs and veins: These include branches of the midvein and also veins which run parallel to it and may be the same size or more commonly smaller than the midvein. Species having protruding midribs (e.g., *Gnaphalium involucratum*) may also have protruding lateral ribs, which, however, always protrude less than the midrib. Some lateral veins are surrounded by collenchyma cells (e.g., *Anaphalis triplinervis*). The palisade mesophyll may be continuous across the lateral ribs (e.g., *Helichrysum filicaule*) or it may be replaced by collenchyma cells (e.g., *Anaphalis triplinervis*). *Helichrysum lanceolatum* has thick-walled bundle-sheaths and thick-walled bundle-sheath extensions (Plate 9, A). All species having sclerenchyma caps on the midvein also have sclerenchyma caps on the lateral veins.

Spaces: A space underneath the abaxial epidermis, other than substomatal chambers, is characteristic of *Raoulia grandiflora* (Plate 15, E; Plate 16, A). Several leaves of different plants were handsectioned in order to establish that the detaching of the epidermis is not caused by the embedding resin. The complete detachment of the mesophyll may be brought about even by hand-sectioning, but indentations of the mesophyll between the bundles clearly leave spaces between the epidermis and the mesophyll.

Secretory ducts: *Haastia pulvinaris* is the only species examined which has secretory ducts (Plate 8, C). These secretory ducts lie on the abaxial side of most of the bundles and are lined with epithelial cells.

Cellular inclusions: Tannin cells are abundant in all species. Neither crystals nor silica bodies were observed, although calcium oxalate crystals have been recorded previously for *Raoulia bryoides* (Hauri, 1916).

Sclerenchyma and thick-walled cells of the mesophyll: Sclerenchyma were not found in the mesophyll. Genus "Z" has a few cells with wall thickening dispersed in the mesophyll (Plate 18, D and E). (It has to be stressed again that sections through the middle of the leaf blade are here compared. The mesophyll of the leaves of *Helichrysum depressum*, for example, is completely sclerenchymatous at the lamina base (Plate 11, A,B). The base of the lamina of *Raoulia* "L" has even a sclerenchymatous epidermis, but these features were not included in the comparison.)

Table 2.2 shows the distribution of selected anatomical characters regarded as taxonomically useful.

Table 2.2. Selected anatomical characters.

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A. keriensis	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	-	+	-	-	2
A. rupestris	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	-	+	-	-	2
A. subrigida	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	-	+	-	-	2
A. trinervis	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	-	+	-	-	2
A. triplinervis	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
C. aculeata	dorsiventral	ab	ab=ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
C. fulvida	dorsiventral	ab	ab<ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
C. leptophylla	dorsiventral	ab	ab<ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
C. longifolia	dorsiventral	ab	ab=ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
E. catipes	equifacial	ab=ad	ab=ad	ab=ad	-	-	-	oval	-	-	-	-	-	1
E. meredithae	dorsiventral	ab	ab<ad	ab=ad	+	-	-	rod	-	?	+	-	-	1
E. planchonii	dorsiventral	ab	ab<ad	ab=ad	+	-	-	oval	-	-	+	-	-	1
E. sinclairii	dorsiventral	ab	ab=ad	ab=ad	-	small	-	rod	-	+	+	-	-	1
G. involucratum	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
G. mackayi	dorsiventral	ab>ad	ab=ad	ab=ad	-	small	-	oval	-	+	+	-	-	1
G. nitidulum	dorsiventral	ab>ad	ab=ad	ab=ad	-	small	-	oval	-	+	+	-	-	1
G. traversii	dorsiventral	ab>ad	ab<ad	ab<ad	-	small	-	rod	-	+	+	-	-	1
G. umbricola	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
Ha. pulvinaris	dorsiventral	ab	ab=ad	ab=ad	+	-	-	rod	-	?	+	-	+	1
Ha. sinclairii	dorsiventral	ab	ab=ad	ab<ad	+	-	-	rod	-	?	+	-	-	1
He. backhousii	dorsiventral	ab	ab<ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
He. bellidioides	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	-	-	-	-	1
He. coralloides	dorsiventral	ad	ab>ad	ab>ad	+	-	-	rod	+	-	-	+	-	1
He. depressum	dorsiventral	ad	ab>ad	ab>ad	+	-	-	rod	+	-	-	?	-	1
He. dimorphums	dorsiventral	ad	ab>ad	ab>ad	+	-	-	rod	+	-	-	-	-	1
He. dimorphumn	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
He. filicaule	dorsiventral	ab>ad	b<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
He. intermedium	dorsiventral	ad	ab>ad	ab>ad	+	-	-	rod*	+	-	-	+	-	1
He. lanceolatum	dorsiventral	ab	ab=ad	ab<ad	+	-	-	rod*	-	+	+	-	-	1
He. obcordatum	dorsiventral	ab	ab<ad	ab<ad	+	-	-	rod	-	+	+	-	-	1
He. parvifolium	dorsiventral	ad	ab>ad	ab>ad	+	-	-	rod	+	-	-	+	-	1
L. grandiceps	equifacial	ab<ad	ab=ad	ab>ad	-	medium	-	rod	-	-	-	-	-	1
L. leontopodium	equifacial	ab<ad	ab=ad	ab>ad	-	medium	-	oval	-	-	-	+	-	1
L. "Marlborough"	equifacial	ad	ab=ad	ab>ad	-	medium	-	oval	-	-	-	+	-	1
L. "Peel"	equifacial	ab=ad	ab=ad	ab>ad	-	medium	-	oval	-	-	-	+	-	1
Pseudognaphalium	dorsiventral	ab=ad	ab=ad	ab=ad	+	-	-	rod	-	+	+	-	-	1
Pterygopappus	equifacial	ad	ab<ad	ab>ad	-	-	+	-	-	-	-	-	-	1
R. bryoides	equifacial	ab=ad	ab=ad	ab=ad	-	-	+	oval	-	-	-	+	-	1
R. cinerea	equifacial	ab<ad	ab=ad	ab=ad	-	small	-	oval	-	+	+	-	-	1
R. eximia	equifacial	ab=ad	ab=ad	ab=ad	-	-	+	oval	-	-	-	+	-	1
R. glabra	equifacial	ab=ad	ab=ad	ab<ad	-	large	-	oval	-	-	-	-	-	1
R. grandiflora	equifacial	ad	ab<ad	ab>ad	-	-	+	oval	-	-	-	+	-	1
R. hectori	equifacial	ab=ad	ab=ad	ab=ad	-	-	+	oval	-	-	-	+	-	1
R. hookeri	equifacial	ab=ad	ab=ad	ab=ad	-	large	-	oval	-	-	-	-	-	1
R. "L"	equifacial	ab=ad	ab=ad	ab=ad	-	-	+	oval	-	-	-	+	-	1
R. "M"	dorsiventral	ab	ab<ad	ab<ad	+	-	-	oval	-	+	-	-	-	1
R. petriensis	dorsiventral	ad	ab>ad	ab>ad	+	-	-	oval	+	-	-	-	-	1
R. tenuicaulis	equifacial	ab=ad	ab=ad	ab=ad	-	large	-	oval	-	-	-	-	-	1
Genus "Z"	equifacial	ab=ad	ab=ad	ab=ad	-	small	-	rod*	-	+	?	-	-	1

Key:
A lamina
B stomata
C epidermal thickness
D cuticle thickness
E spongy parenchyma
F middle cells
G mesophyll poorly differentiated
H shape of palisade cells; * = Intermediate between oval and rod-shaped
I palisade parenchyma only on the abaxial side
J protruding midrib
K abaxial collenchyma
L sclerenchyma caps
M secretory ducts
N number of bundle-sheath layers
ad = adaxial, ab = abaxial, rod = rod-shaped.

2.4.2. Leaf anatomy descriptions

Subtribe Gnaphaliinae

Lamina bifacial, dorsiventral to equifacial, 100-400 μm thick. **Cuticle** 0-40 μm thick, either of equal thickness on both surfaces or thicker on the abaxial or the adaxial surface, sometimes thickened at the margin. **Epidermis** of 1 layer of isodiametric or oval or rectangular cells; cells of the adaxial and abaxial surface of similar or dissimilar size; cell height 10-50 μm; stomata either in equal or unequal numbers on both surfaces or present on only one of the surfaces; guard cells 5-10 μm in diameter, subsidiary cells not differentiated from other epidermal cells. **Hypodermis** absent. **Leaf-margin** rounded or rarely pointed. **Mesophyll** variable. **Midrib** with a collateral vein surrounded by a parenchymatous bundle-sheath (in *Helichrysum lanceolatum* thick-walled); sclerenchyma caps, collenchyma cells or other thick-walled cells occasionally present. **Lateral ribs** similar or dissimilar to midrib; major veins of equal size to midvein or smaller. **Tannins** common in all species. **Crystals** absent. **Silica bodies** absent.

Specific taxa

Anaphalis keriensis, A. rupestris, A. subrigida, A. trinervis

(Plate 1, A,B,C,D,E,F)

Lamina dorsiventral, 200-400 μm thick. **Cuticle** 0-15 μm thick, thicker on the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 25-50 μm, abaxially 10-25 μm; stomata confined to the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 50-100 μm thick; palisade cells in 1-2 rows, rod-shaped, 30-100 μm long and 10-40 μm wide; spongy tissue 150-200 μm thick, with numerous airspaces; spongy parenchyma cells elongated parallel to the leaf surface, up to 80 μm long and 25 μm wide, smaller towards the abaxial epidermis. **Leaf-margin** rounded or pointed, with very thick cuticle; palisade cells restricted to the adaxial side. **Midrib**

not protruding; midvein 90-200 μm in diameter, surrounded by a several cell layers thick, parenchymatous bundle-sheath; cells of the bundle-sheath smaller than cells of the collenchyma; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 60-120 μm in diameter, equidistant from both surfaces; collenchyma cells surrounding the veins and extending to the epidermis above and below.

Anaphalis keriensis (Plate 1, A)

Cuticle less than 5 μm thick. **Epidermis** with cells 25-30 μm high adaxially, 10-15 μm abaxially. **Mesophyll** clearly differentiated into palisade parenchyma 50 μm thick, with palisade cells 30-50 μm long and 10-20 μm wide, and into spongy parenchyma 150 μm thick with cells up to 80 μm long. **Leaf-margin** rounded. **Midrib** indented at the adaxial side; midvein 90-110 μm in diameter, equidistant from both surfaces.

Anaphalis rupestris (Plate 1, C,F)

Cuticle less than 5 μm thick. **Epidermis** with cells 50 μm high adaxially, 10-15 μm abaxially. **Mesophyll** clearly differentiated into palisade parenchyma 60 μm thick, with palisade cells 50-100 μm long and 20-40 μm wide, and into spongy parenchyma 200 μm thick with cells up to 70 μm in diameter. **Leaf-margin** rounded. **Midrib** with midvein 180-200 μm in diameter, closer to the adaxial than to the abaxial surface; cells on the adaxial side of the vein thick-walled.

Anaphalis subrigida (Plate 1, D,E)

Cuticle at the abaxial surface less than 5 μm thick, but 5-15 μm thick at the adaxial surface. **Epidermis** with cells 30-40 μm high adaxially, 15-25 μm abaxially. **Mesophyll** clearly differentiated into palisade parenchyma 100 μm thick, with palisade cells 40-50 μm long and 10-20 μm wide, and into spongy parenchyma 200 μm thick with cells up to 60 μm long. **Leaf-margin** pointed. **Midrib** with midvein approximately 150 μm in diameter, equidistant from both surfaces.

Anaphalis trinervis (Plate 1, B)

Cuticle less than 5 μm thick. **Epidermis** with cells 30-40 μm high adaxially, abaxially 10-15 μm; adaxial epidermis in midrib with 4 smaller cells. **Mesophyll** clearly differentiated into palisade parenchyma 70 μm thick, with palisade cells of 30-70 μm length and 10-20 μm width, and into spongy parenchyma 200 μm thick with cells up to 60 μm long. **Leaf-margin** pointed. **Midrib** indented at the adaxial side; midvein 120-150 μm in diameter, closer to the abaxial than to the adaxial surface.

Anaphalis triplinervis (Plate 2, A,B,C,D)

Lamina dorsiventral, approximately 250 μm thick. **Cuticle** at the adaxial side 5-10 μm, at the abaxial side less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells at the adaxial and irregular cells at the abaxial side; cell height adaxially 30-40 μm, abaxially 10-15 μm; abaxial cells of the midrib almost the same size as adaxial cells; stomata confined to the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 80 μm thick; palisade cells in 1-2 rows, rod-shaped, 40-60 μm long and 20-30 μm wide adjacent to the epidermis, smaller in the second row; spongy tissue in 5-6 rows, 110 μm thick, cells elongated parallel to the leaf surface, 25-40 μm long, smaller towards the abaxial epidermis. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding abaxially 400-500 μm, but nearly level adaxially; midvein approximately 200 μm in diameter, much closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** protruding; major veins 120 μm in diameter, much closer to the adaxial than to the abaxial surface; collenchyma cells surrounding the vein and extending to the epidermis above and below.

Cassinia aculeata, C. longifolia

(Plate 2, E,F; Plate 3, A,B)

Lamina dorsiventral, 130-170 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** at the adaxial surface with regular, oval cells, at the abaxial surface with regular, isodiametric cells; cell height adaxially and abaxially 10-15 μm; stomata confined to the abaxial surface; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 70-110 μm thick; palisade cells in 1-2 rows, rod-shaped, 20-60 μm long and 10-15 μm wide adjacent to the epidermis, smaller in the second row; spongy tissue loosely arranged, 55-70 μm thick, cells elongated parallel to the leaf surface, 25 μm long, smaller towards the abaxial epidermis. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding approximately 200 μm abaxially; midvein surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis below; palisade cells continuous; sclerenchyma caps absent. **Lateral ribs** with veins almost equidistant from both surfaces; mesophyll normal.

Cassinia aculeata (Plate 2, E,F; Plate 3, A,B)

Epidermis with stomata extremely raised above the normal epidermal cells. **Midrib** very much indented opposite the protruding side; midvein 80 μm in diameter, much closer to the adaxial than to the abaxial surface. **Lateral ribs** not protruding; major veins 30 μm in diameter.

Cassinia longifolia

Epidermis with stomata raised above the normal epidermal cells. **Midrib** indented opposite the protruding side; midvein 140-220 μm in diameter, equidistant from both surfaces. **Lateral ribs** protruding; major veins 110 μm in diameter.

Cassinia fulvida, C. leptophylla, Helichrysum backhousii

(Plate 3, C,D,E,F)

Lamina dorsiventral, 200-350 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells at the adaxial surface and irregularly shaped cells at the abaxial side; cell height adaxially 15-20 μm, abaxially 10-15 μm; stomata confined to the abaxial surface, raised above the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 80-200 μm thick; palisade cells in 2-6 rows, rod-shaped, 20-80 μm long and 10-30 μm wide; length of the palisade cells variable depending on number of palisade rows; spongy tissue loosely arranged, 70-110 μm thick, cells elongated parallel to the leaf surface, 10-20 μm long. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding 100-150 μm abaxially, but nearly level adaxially; midvein 70-160 μm in diameter, much closer to the abaxial than to the adaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis below; palisade cells continuous; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 20 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Cassinia fulvida (Plate 3, D,F)

Lamina 300 μm thick. **Mesophyll** with palisade tissue in 2-6 rows, 80-190 μm thick; palisade cells 20-80 μm long and 10-20 μm wide; spongy tissue 70-100 μm thick. **Midvein** 70 μm in diameter.

Cassinia leptophylla (Plate 3, C,E)

Lamina 200 μm thick. **Mesophyll** with palisade tissue in 2-3 rows, 100 μm thick; palisade cells 40-50 μm long and 20-30 μm wide; spongy tissue 80 μm thick. **Midvein** 70 μm in diameter.

Helichrysum backhousii

Lamina 350 μm thick. **Mesophyll** with palisade cells in 2-6 rows, 200 μm thick; palisade cells 20-80 μm long and 10-20 μm wide; spongy tissue 110 μm thick. **Midvein** 90-160 μm in diameter.

Ewartia catipes (Plate 4, A,B,C,D)

Lamina almost equifacial, 130 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 10-15 μm and abaxially 8-10 μm; equal numbers of stomata on both surfaces, slightly raised above the normal epidermal cells; substomatal chambers small. **Mesophyll** poorly differentiated into a 30 μm thick adaxial layer of 1-2 rows of palisade-like, long-oval cells, a 55 μm thick middle layer of 2-3 rows of broad-oval cells and a 30 μm thick abaxial layer of 1 row of palisade-like, long-oval cells; palisade-like cells compactly arranged, 15-25 μm long and 10-15 μm wide; oval cells in the middle layer 15-20 μm long and 10 μm wide. **Leaf-margin** rounded; palisade-like cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein 30-40 μm in diameter, equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells absent; mesophyll normal; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 30-40 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Ewartia meredithae (Plate 4, E,F; Plate 5, A,B)

Lamina dorsiventral, 170 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 15-20 μm, abaxially 10-15 μm; stomata confined to the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 60-80 μm thick; palisade cells in 1-2 rows, rod-shaped, 60-80 μm long and 20-25 μm wide; spongy tissue loosely arranged, 95 μm thick, cells elongated parallel to the leaf surface, 30 μm long, smaller towards the abaxial epidermis. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** not

protruding; midvein 100 μm in diameter, closer to the abaxial than to the adaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis below; palisade cells continuous; sclerenchyma caps absent; cells on both sides of the midvein thick-walled. **Lateral ribs** not protruding; major veins 40 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Ewartia planchonii (Plate 5, C,D)

Lamina dorsiventral, 130-160 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 20-25 μm, abaxially 5-10 μm; stomata confined to the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 40 μm thick; palisade-like, oval cells in 1-2 rows, 30-40 μm long and 15-20 μm wide; spongy tissue loosely arranged, in 4 layers, 70 μm thick, cells elongated parallel to the leaf surface, 25 μm long, smaller towards the epidermis. **Leaf-margin** rounded; palisade-like cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein 40-80 μm in diameter, closer to the abaxial than to the adaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis below; palisade cells continuous; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 55 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Ewartia sinclairii (Plate 5, E,F; Plate 6, A,B,D)

Lamina almost dorsiventral, 120-140 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular, isodiametric or oval cells; cell height adaxially and abaxially 10-20 μm; stomata confined to the abaxial surface, raised above the normal epidermal cells; substomatal chambers small. **Mesophyll** differentiated into 1-2 rows of palisade parenchyma, 2-3 rows of medium-sized round middle cells and 1-2 rows of small, round cells; palisade tissue confined to the adaxial side, compactly arranged, 65-110 μm thick; palisade cells 20-40 μm long and 10-15 μm wide; tissue of

round cells in the middle of the leaf 40 μm thick, cells 20-30 μm in diameter; tissue of small round cells at the abaxial side, 20 μm thick, cells 8-20 μm in diameter. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding 130 μm abaxially, but level adaxially; midvein 75 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein; palisade cells continuous, but shorter; sclerenchyma caps absent. **Lateral ribs** protruding; major veins 40 μm in diameter, slightly closer to the adaxial than to the abaxial surface; collenchyma cells surrounding the vein, not extending to the epidermis above and below.

Gnaphalium involucratum, G. umbricola (Plate 6, C,E,F)

Lamina dorsiventral, approximately 130-160 μm thick. **Cuticle** at the adaxial side 5-15 μm thick, less than 5 μm on the abaxial side, slightly thicker above the midvein. **Epidermis** with regular isodiametric or oval cells at the adaxial side and irregular cells at the abaxial side; cell height adaxially approximately 30 μm, abaxially 5-10 μm, cells above the midvein with almost the same size as adaxial epidermal cells; stomata confined to the adaxial surface, at the same level as the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 30-55 μm thick; palisade cells in 1 row, rod-shaped, 35-55 μm long and 5-25 μm wide; spongy tissue 30-55 μm thick, cells elongated parallel to the leaf surface, 10-20 μm long, smaller towards the epidermis. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding 400 μm abaxially, but level adaxially; midvein 110-150 μm in diameter, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** slightly protruding; major veins 40 μm in diameter, closer to the abaxial than to the adaxial surface; mesophyll normal.

Gnaphalium involucratum

Lamina 160 μm thick. **Cuticle** at the adaxial side 15 μm thick. **Mesophyll** with palisade tissue 55 μm thick; palisade cells approximately 35-55 μm long and 20-25 μm wide. **Midrib** with midvein 110-150 μm, closer to the adaxial than to the abaxial surface.

Gnaphalium umbricola (Plate 6, C,E,F)

Lamina 130 μm thick. **Cuticle** at the adaxial side 5-10 μm thick. **Mesophyll** with palisade tissue 30-55 μm thick; palisade cells 40-55 μm long and 5-20 μm wide. **Midrib** with midvein 130-150 μm, equidistant from both surfaces.

Gnaphalium mackayi (Plate 7, A,B,C)

Lamina almost dorsiventral, approximately 130 μm thick. **Cuticle** less than 5 μm. **Epidermis** with regular isodiadiametric or oval cells; cell height adaxially and abaxially 10-20 μm; stomata on both surfaces, but more numerous on the abaxial surface, almost level with the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** poorly differentiated into 1-2 rows of palisade-like cells on the adaxial side, 2-3 rows of medium-sized round to broadly oval cells in the middle and 2-3 rows of small round cells on the abaxial side; palisade tissue compactly arranged, 30 μm thick; palisade-like cells oval, 20-30 μm long and 10-20 μm wide; tissue of round middle cells 50 lm thick, cells approximately 25 lm long; abaxial tissue of small round cells 30 lm thick, cells 10 lm in diameter. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding abaxially 50 μm; midvein 70 μm in diameter, almost equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 55 μm in diameter, equidistant from both surfaces; mesophyll normal.

Gnaphalium nitidulum (Plate 7, D,E,F)

Lamina almost equifacial, approximately 130 μm thick. **Cuticle** less than 5 μm. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10-20 μm; stomata on both surfaces, but more numerous on the abaxial surface, almost level with the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** poorly differentiated into 1 row of palisade-like cells on the adaxial side, 3 rows of medium-sized round to broadly oval cells in the middle of the leaf and 1 row of palisade-like cells at the abaxial side; adaxial tissue compactly arranged, 35 μm thick; palisade-like cells oval, 25-35 μm long and 10-20 μm wide; central tissue 40 μm thick, cells approximately 25 μm long; abaxial tissue 25 μm thick, cells 10-35 μm long and 10-20 μm wide. **Leaf-margin** rounded. **Midrib** protruding 60 μm; midvein approximately 55 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade-like cells absent; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 30 μm in diameter, closer to the abaxial than to the adaxial surface; mesophyll normal.

Gnaphalium traversii (Plate 8, A,B)

Lamina almost dorsiventral, approximately 130 μm thick. **Cuticle** less than 5 μm, slightly thicker at the adaxial side and at the margins than at the abaxial side. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 10-25 μm, abaxially approximately 10 μm; stomata on both surfaces, but more numerous on the abaxial surface, almost level with the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** differentiated into 1 row of palisade cells on the adaxial side, 2-3 rows of round to broadly oval cells in the middle and 2-3 rows of small round cells on the abaxial side; palisade tissue compactly arranged, approximately 20 μm thick; palisade cells approximately 30 μm long and 10 μm wide; central tissue 45 μm thick, cells approximately 25 μm long; abaxial tissue 25 μm thick, cells 10 μm in diameter. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding 115 μm abaxially but nearly level adaxially; midvein approximately 75-95 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath;

collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 40 μm in diameter, closer to the abaxial than to the adaxial surface; mesophyll normal.

Haastia pulvinaris, H. sinclairii (Plate 8, C,D,E,F)

Lamina dorsiventral, 220-330 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 25-30 μm, abaxially 15-30 μm; stomata on the abaxial side, raised above the level of the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; layer of palisade tissue confined to the adaxial side, compactly arranged, 80-100 μm thick; palisade cells 35-85 μm long and 15-30 μm wide, in 1-2 rows; spongy tissue 40-80 μm thick; upper part of the leaf crenulate. **Leaf-margin** rounded. **Midrib** greatly protruding abaxially, indented at the adaxial side; midvein 40-70 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** similar to midrib.

Haastia pulvinaris (Plate 8, C,D,E)

Epidermis with cell height abaxially 25-30 μm; stomata in the crenulate part of the leaf on both surfaces. **Mesophyll** with palisade cells becoming shorter towards the leaf tip. **Resin canals** situated abaxially to the veins (Plate 8, C,c).

Haastia sinclairii (Plate 8, F)

Epidermis with cell height abaxially 15 μm; stomata confined to the abaxial surface. **Resin canals** absent.

Helichrysum bellidioides (Plate 9, C,D,E,F)

Lamina dorsiventral, approximately 250 μm thick. **Cuticle** less than 5 μm thick, thicker on the adaxial than on the abaxial side. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 20-40 μm, abaxially 10-15 μm; stomata confined to the abaxial surface. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 80 μm thick; palisade cells in 1-2 rows, oval, 35-70 μm long and 10-20 μm wide; spongy tissue loosely arranged, 120-150 μm thick, cells in 3 rows, elongated parallel to the leaf surface, 15-25 μm long. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein 100 μm in diameter, closer to the abaxial than to the adaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein; palisade cells continuous; sclerenchyma caps absent; cells on the adaxial side of the vein thick-walled. **Lateral ribs** with major veins 40 μm in diameter, much closer to the abaxial surface than to the adaxial surface; mesophyll normal.

Helichrysum coralloides, H. intermedium, H. parvifolium

(Plate 10, A,B,C)

Lamina inverse-dorsiventral, 130-200 μm thick. **Cuticle** less than 5 μm thick at the adaxial surface, 15-40 μm thick at the abaxial surface. **Epidermis** with regular isodiametric or oval cells on the adaxial surface and regular, rectangular cells on the abaxial surface; cell height adaxially 5-10 μm, abaxially 15-20 μm; stomata confined to the adaxial surface, raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the abaxial side, compactly arranged,40-80 μm thick; palisade cells in 1-3 rows, rod-shaped, 25-55 μm long and 10-20 μm wide; spongy tissue 40-55 μm thick, confined to the adaxial side, cells elongated, in 3 rows parallel to the leaf surface, 8-20 μm long, smaller towards the surface. **Leaf-margin** rounded; palisade cells absent. **Midrib** not protruding; midvein approximately 100 μm in diameter, much closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps at the abaxial side of the midvein. **Lateral ribs** not protruding; major

veins 30 μm in diameter, much closer to the adaxial surface than to the abaxial surface; mesophyll normal; (lateral ribs in *H. parvifolium* absent).

Helichrysum depressum (Plate 10, D,E,F)

Lamina inverse-dorsiventral, approximately 120 μm thick. **Cuticle** less than 5 μm thick, but thicker at the abaxial than the adaxial surface. **Epidermis** with regular isodiametric or oval cells at the adaxial surface and regular, rectangular cells at the abaxial surface; cell height adaxially 5-10 μm, abaxially 15-20 μm; stomata confined to the adaxial surface, extremely raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the abaxial side, compactly arranged, 40 μm thick; palisade cells in 1-3 rows, rod-shaped, 35-40 μm long and 10-15 μm wide; spongy tissue confined to the adaxial side, loosely arranged, 55 μm thick, cells elongated parallel to the leaf surface, variable in size. **Leaf-margin** rounded; palisade cells restricted to the abaxial side. **Midrib** not protruding; midvein 40-50 μm in diameter, much closer to the adaxial than to the abaxial side, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent; cells at the adaxial side of the vein thick-walled. **Lateral ribs** not protruding; major veins 30 μm in diameter, much closer to the adaxial than to the abaxial surface; mesophyll normal.

Helichrysum dimorphum

scale-like leaf: (Plate 11, C)

Lamina inverse-dorsiventral, approximately 110 μm thick. **Cuticle** less than 5 μm thick at the adaxial side, but at the abaxial side 15-20 μm thick. **Epidermis** with regular isodiametric or oval cells at the adaxial surface and regular, rectangular cells at the abaxial surface; cell height adaxially 5-8 μm, abaxially 10-20 μm; stomata confined to the adaxial surface, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the abaxial side, compactly arranged, 55 μm thick; palisade cells in 1-2 rows, rod-shaped, 30-50 μm long and 10-15 μm wide

adjacent to the epidermis; spongy tissue 30-40 μm thick, loosely arranged, confined to the adaxial side, cells elongated parallel to the leaf surface, 10-15 μm long and 20-40 μm wide, smaller towards the surface. **Leaf-margin** rounded; palisade cells restricted to the abaxial side. **Midrib** not protruding; midvein 40 μm in diameter, much closer to the adaxial than to the abaxial side, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent; cells at the adaxial side of the vein thick-walled. **Lateral ribs** not protruding; major veins 20 μm in diameter, much closer to the adaxial than to the abaxial surface; mesophyll normal.

normal leaf: (Plate 11, D)

Lamina dorsiventral, approximately 100 μm thick. **Cuticle** less than 5 μm thick at the abaxial surface, thicker at the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 8-15 μm, abaxially 10-15 μm; stomata confined to the abaxial surface, level with the normal epidermal cells; substomatal chambers small. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 55 μm thick; palisade cells in 1-2 rows, rod-shaped, 30-50 μm long and 10-15 μm wide adjacent to the epidermis; spongy tissue 30-40 μm thick, loosely arranged, confined to the adaxial side, cells elongated parallel to the leaf surface, 10-15 μm long and 20-40 μm wide, smaller towards the surface. **Leaf-margin** rounded, palisade cells restricted to the adaxial side. **Midrib** protruding abaxially 120 μm, but nearly level adaxially; midvein 30 μm in diameter, closer to the abaxial than to the adaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein; palisade cells continuous; sclerenchyma caps absent. **Lateral ribs** with major veins 20 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Helichrysum filicaule (Plate 11, E,F)

Lamina dorsiventral, approximately 140 μm thick. **Cuticle** 10 μm thick at the adaxial surface, less than 5 μm thick at the abaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 20-25 μm, abaxially 10 μm; stomata on both sides, but more numerous on the abaxial surface; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 35 μm thick; palisade cells in 1-2 rows, approximately 25 μm long and 10-15 μm wide; spongy tissue 45 μm thick, in 3 rows, loosely arranged, cells elongated parallel to the leaf surface, 15 μm in long. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding abaxially 150 μm, nearly level adaxially; midvein 90 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis below; palisade cells continuous; sclerenchyma caps absent. **Lateral ribs** with major veins 40 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Helichrysum lanceolatum (Plate 9, A,B)

Lamina dorsiventral, approximately 160 μm thick. **Cuticle** less than 5 μm thick, slightly thicker on the adaxial than on the abaxial surface. **Epidermis** with regular, round to oval cells; cell height adaxially 10 μm, abaxially 5 μm; stomata confined to the abaxial surface, raised above the level of the normal epidermal cells; substomatal chambers small. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 30 μm thick; cells in 1 row, oval, 20-30 μm long and 10-20 μm wide; spongy tissue compactly arranged, 100 μm thick, cells in 3-4 rows, 30-50 μm in diameter, smaller towards the surface. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding abaxially 140 μm; midvein 140-180 μm, equidistant from both surfaces, surrounded by a single-layered thick-walled bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; palisade cells absent; sclerenchyma caps absent. **Lateral ribs** with major veins 110 μm in diameter, equidistant from both surfaces; thick-walled bundle-sheath extensions on both sides of the veins.

Helichrysum obcordatum (Plate 11, A,B)

Lamina dorsiventral, approximately 160-190 μm thick. **Cuticle** less than 5 μm thick at the abaxial surface, 10 μm thick at the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 20 μm, abaxially 10 μm; stomata confined to the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 80 μm thick; palisade cells in 1-2 rows, rod-shaped, 50-90 μm long and 15-20 μm wide; spongy tissue 60 μm thick, cells elongated parallel to the leaf surface, in 2-3 rows, 40 μm long and 20 μm wide, smaller towards the surface. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding abaxially 120 μm, but nearly level adaxially; midvein 80 μm in diameter, equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein; palisade cells continuous. **Lateral ribs** not protruding; major veins 40 μm in diameter, much closer to the abaxial surface than to the adaxial ; mesophyll normal.

Leucogenes grandiceps (Plate 12, C,D)

Lamina almost equifacial, approximately 120-150 μm thick. **Cuticle** less than 5 μm thick at the adaxial surface, but 10 μm thick at the abaxial surface, thicker at the margin. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10-20 μm; stomata more numerous on the adaxial than on the abaxial surface, slightly raised above the normal epidermal cells; substomatal chambers small. **Mesophyll** clearly differentiated into a 40 μm thick adaxial layer of 1-2 rows of compactly arranged palisade cells, a 65 μm thick middle layer of 2-3 rows of medium-sized, polygonal cells, and a 20 μm thick abaxial layer of 1-2 rows of small, round cells; palisade cells 15-40 μm long and 8-10 μm wide, medium-sized, polygonal cells 25-45 μm in diameter, abaxial round cells 15-20 μm in diameter. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein approximately 40-55 μm in diameter, almost equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 40-55 μm in diameter; mesophyll normal.

Leucogenes leontopodium, Leucogenes "Peel", *Leucogenes* "Marlborough" (Plate 12, E,F; Plate 13, A,B)

Lamina almost equifacial, 220-270 μm thick, becoming gradually thinner towards the margin. **Cuticle** less than 5 μm thick, slightly thicker at the abaxial side and at the margin than at the adaxial side. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 8-25 μm, abaxially 15-25 μm; stomata level with the normal epidermal cells; substomatal chambers small. **Mesophyll** poorly differentiated into a 40-65 μm thick adaxial layer of 1-5 rows of palisade-like, oval cells, loosely arranged, a 110-140 μm thick middle layer of 2-4 rows of medium-sized round cells, and a 15-30 μm thick abaxial layer of 1 row of small round cells; adaxial oval cells 15-55 μm long and 15-30 μm wide, medium-sized round cells 40-50 μm in diameter, abaxial round cells approximately 15 μm in diameter. **Leaf-margin** rounded; long-oval cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein approximately 150 μm in diameter, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps at both sides of the vein. **Lateral ribs** not protruding; major veins 140 μm in diameter, closer to the adaxial than to the abaxial surface; mesophyll normal.

Leucogenes leontopodium (Plate 12, E,F; Plate 13, B)

Lamina approximately 270 μm thick. **Epidermis** with stomata more numerous on the adaxial than on the abaxial surface. **Mesophyll** poorly differentiated into a 40 μm thick adaxial layer of 1-2 rows of oval cells, a 130 μm thick middle layer of 2-3 rows of medium-sized round cells, and a 15-30 μm thick abaxial layer of small round cells; adaxial oval cells 15-55 μm long and 15-30 μm wide.

Leucogenes "Marlborough"

Lamina approximately 255 μm thick. **Epidermis** with stomata confined to the adaxial surface. **Mesophyll** poorly differentiated into a 55-65 μm thick layer of 3-5 rows of oval cells, a 110 μm thick middle layer of 3-4 rows of medium-sized round cells, and a 15 μm thick abaxial layer of 1 row of small round cells; adaxial oval cells 15 μm long and 20 μm wide.

Leucogenes "Peel" (Plate 13, A)

Lamina approximately 220 μm thick. **Epidermis** with only a few stomata on both surfaces. **Mesophyll** poorly differentiated into a 40 μm thick adaxial layer of 1-2 rows of oval cells, a 140 μm middle layer of 2-3 rows of medium-sized round cells, and a 15 μm thick abaxial layer of 1 row of small round cells; adaxial oval cells 15-55 μm long and 15-30 μm wide.

Pseudognaphalium luteoalbum (Plate 13, C,D,E,F)

Lamina dorsiventral, approximately 110 μm thick. **Cuticle** less than 5 μm thick, slightly thicker at the margin than on both surfaces. **Epidermis** with cells of very irregular size and shape; cell height 10-40 μm; stomata in equal numbers on both surfaces, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** clearly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, compactly arranged, 25-40 μm thick; palisade cells in 1 row, rod-shaped, 35-50 μm long and 10-20 μm wide; spongy tissue in 3-4 rows, 40 μm thick, loosely arranged, cells elongated parallel to the leaf surface, 10-20 μm long. **Leaf-margin** rounded; palisade cells restricted to the adaxial side. **Midrib** protruding approximately 300 μm abaxially but nearly level adaxially; midvein 85-110 μm in diameter, much closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; collenchyma cells surrounding the vein and extending to the epidermis above and below; sclerenchyma caps absent. **Lateral ribs** not protruding, 45 μm in diameter, closer to the abaxial than to the adaxial surface; mesophyll normal.

Pterygopappus lawrencii (Plate 14, A,B)

Lamina almost homogeneous, approximately 155 μm thick, becoming gradually thinner towards the margins. **Cuticle** less than 5 μm, slightly thicker on the abaxial than the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 15 μm, abaxially 20 μm; stomata confined to the adaxial surface, slightly raised above the normal epidermal cells; substomatal chambers very small; one adaxial epidermal cell in the midrib enlarged; epidermis in places two cells deep. **Mesophyll** undifferentiated, very loosely arranged, with cells more or less round at the centre, becoming oval towards both surfaces; cells 15-35 μm in diameter. **Leaf-margin** rounded. **Midrib** not protruding, midvein 25-35 μm in diameter, closer to the adaxial than to the abaxial surface; midvein surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 25-35 μm in diameter, closer to the adaxial than to the abaxial side; mesophyll normal.

Raoulia bryoides, R. eximia, R. hectori, R. "L"

(Plate 14, C,D,E,F)

Lamina equifacial, 110-170 μm thick, becoming gradually thinner towards the margins. **Cuticle** less than 5 μm. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10 μm; stomata in equal numbers on both surfaces, slightly raised above the normal epidermal cells; substomatal chambers small. **Mesophyll** poorly differentiated into oval cells adjoining the epidermal layers and round cells in the lamina centre; oval cells 10 μm wide and 25 μm long at the adaxial side, 20 μm long at the abaxial side, round cells 10 μm in diameter, compactly arranged. **Leaf-margin** rounded, mesophyll cells oval. **Midrib** not protruding; midvein diameter variable depending on size of sclerenchyma caps; sclerenchyma caps at the adaxial side of the vein, at the abaxial side only in *Raoulia bryoides*, sometimes the whole lamina sclerenchymatous; midvein surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal. **Lateral ribs** not protruding; major veins half the size of midvein, sclerenchymatous; mesophyll normal.

Raoulia cinerea (15, A,B,C,D)

Lamina almost equifacial, 160-200 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10-15 μm; abaxial epidermal cell walls slightly thicker at the margin and at the abaxial side of the midrib; stomata on both surfaces, but more stomata on the adaxial surface; stomata level with the normal epidermal cells; substomatal chambers small. **Mesophyll** differentiated into a 80 μm thick adaxial layer of 2-3 rows of palisade-like, oval, cells, a 40 μm thick middle layer of 2 rows of small, round cells and a 55 μm thick layer of 3-6 rows of oval, cells; oval cells adjacent to the epidermis at the adaxial side approximately 15-25 μm long and 10-20 μm wide, at the abaxial side 15-20 μm long and 10 lm wide; round middle cells approximately 20-40 μm in diameter. **Leaf-margin** rounded; oval cells continuous around the periphery of the leaf. **Midrib** protruding approximately 10 μm at the abaxial side; midvein 40-60 μm in diameter, equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; 2-3 rows of collenchyma cells between the bundle-sheath and the abaxial surface, but otherwise no specialised tissue surrounding the vein; palisade cells continuous. **Lateral ribs** protruding; major veins 40 μm in diameter, closer to the abaxial than to the adaxial surface; mesophyll normal.

Raoulia glabra (Plate 16, C,D)

Lamina almost equifacial, approximately 255 μm thick, becoming gradually thinner towards the margins. **Cuticle** at the adaxial surface 8-10 μm thick, at the abaxial surface less than 5 μm. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 10-20 μm, abaxially 15-20 μm; stomata in equal numbers on both surfaces, level with the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** differentiated into a 65 μm thick adaxial layer of 3-4 rows of loosely arranged, small, oval cells, a 85 μm thick middle layer of large, polygonal cells, and a 20 μm thick abaxial layer of 2-3 rows of small, round cells; oval cells 10-35 μm long and 10-15 μm wide, polygonal cells 40-75 μm in diameter. **Leaf-margin** rounded; oval cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein 35 μm in diameter, equidistant from both surfaces; sclerenchyma caps absent; midvein surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal. **Lateral ribs** not protruding; major

veins approximately 30 μm in diameter, almost equidistant from both surfaces; mesophyll normal.

Raoulia grandiflora (Plate 15, E,F; Plate 16, A,B)

Lamina almost equifacial, approximately 270 μm thick but becoming gradually thinner towards the margins; spaces underneath the abaxial epidermis. **Cuticle** less than 5 μm thick, slightly thicker at the abaxial than at the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10-15 μm; stomata only on the adaxial surface, level with the normal epidermal cells; substomatal chambers very small. **Mesophyll** poorly differentiated into 2 to 3 layers of small, oval cells at the adaxial side and 4 layers of small, round cells at the abaxial side; oval cells approximately 30 μm long and 20 μm wide, round cells approximately 20 μm in diameter, compactly arranged. **Leaf-margin** rounded; oval cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein diameter variable depending on amount of sclerenchyma; sclerenchyma cap at the adaxial side of the vein; midvein closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal. **Lateral ribs** not protruding; major veins equal in size to midvein, sclerenchymatous, closer to the abaxial than to the adaxial surface; mesophyll normal.

Raoulia hookeri, R. tenuicaulis

(Plate 16, E,F; Plate 17, A,B)

Lamina almost equifacial, 145-190 μm thick, becoming gradually thinner towards the margins. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 10-20 μm; stomata in equal numbers on both surfaces, level with the normal epidermal cells; substomatal chambers small. **Mesophyll** clearly differentiated into a 30 μm thick adaxial and a 15 μm thick abaxial layer of 1-2 rows of loosely arranged, small, oval cells and a 80 μm thick middle layer of large, polygonal cells; oval cells 15-20 μm long and 15 μm wide, polygonal cells 25-85 μm in diameter. **Leaf-margin** rounded; oval cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein 35-55 μm in diameter, equidistant from

both surfaces; sclerenchyma caps absent; midvein surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal. **Lateral ribs** not protruding; major veins 35-55 μm in diameter; mesophyll normal.

Raoulia "M" (Plate 17, C,E,F)

Lamina dorsiventral, approximately 165 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular, isodiametric or oval cells; cell height adaxially 15 μm, abaxially 10 μm, except cells of the abaxial side of the midrib with almost the same size as the adaxial cells; stomata confined to the abaxial surface, very numerous, very much raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** poorly differentiated into palisade and spongy parenchyma; palisade tissue confined to the adaxial side, 55 μm thick; palisade-like, oval cells in 2-3 rows, 15-20 μm long and 15 μm wide; spongy tissue 80 μm thick, loosely arranged with cells of variable size and shape. **Leaf-margin** rounded; palisade cells continuous around the periphery of the leaf. **Midrib** protruding abaxially approximately 85 μm; midvein 35-40 μm in diameter, situated in the protruding part of the midrib, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 20 μm in diameter, much closer to the abaxial than to the adaxial surface; mesophyll normal.

Raoulia petriensis (Plate 18, A,B)

Lamina inverse-dorsiventral, approximately 145 μm thick. **Cuticle** less than 5 μm thick, slightly thicker at the abaxial than the adaxial surface. **Epidermis** with regular isodiametric or oval cells; cell height adaxially 10 μm, abaxially 15 μm; stomata confined to the adaxial surface, very numerous, slightly raised above the normal epidermal cells; substomatal chambers large. **Mesophyll** differentiated into palisade and spongy parenchyma; palisade tissue confined to the abaxial side, compactly arranged, 40 μm thick; palisade-like, oval cells in 3 rows, 10-25 μm long and 10-20 μm wide; spongy tissue loosely arranged with cells of variable size and shape, becoming smaller towards the surface. **Leaf-margin** rounded; palisade-like cells continuous around the periphery of the leaf. **Midrib** not protruding; midvein approximately 40 μm in

diameter, equidistant from both surfaces, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent. **Lateral ribs** not protruding; major veins 40 μm in diameter, equidistant from both surfaces; mesophyll normal.

Genus "Z" (Plate 18, C,D,E,F)

Lamina equifacial, approximately 265 μm thick. **Cuticle** less than 5 μm thick. **Epidermis** with regular isodiametric or oval cells; cell height adaxially and abaxially 25-35 μm; abaxial cell walls slightly thicker in midrib; stomata on both surfaces in equal numbers, level with the normal epidermal cells; substomatal chambers medium in size. **Mesophyll** differentiated into 2-3 rows of palisade parenchyma on both sides and 2-3 rows of small round cells at the centre; each layer approximately 65 μm thick; palisade cells oval, 15-50 μm long and 10-25 μm wide; round cells 25-35 μm in diameter, some of them thick-walled. **Leaf-margin** rounded; oval cells continuous around the periphery of the leaf. **Midrib** protruding abaxially approximately 140 μm; midvein 65 μm in diameter, closer to the adaxial than to the abaxial surface, surrounded by a single-layered parenchymatous bundle-sheath; mesophyll normal; sclerenchyma caps absent; some cells on the abaxial side of the midvein thick-walled. **Lateral ribs** protruding; major veins 60 μm in diameter, closer to the adaxial than to the abaxial surface; mesophyll normal.

Plate 1: Legends

1 A: *Anaphalis keriensis*, T.S. lamina (L.M.), x 220.

1 B: *Anaphalis trinervis*, T.S. lamina with lateral vein (S.E.M.), x 160.

1 C: *Anaphalis rupestris*, T.S. midrib (L.M.), x 190.

1 D: *Anaphalis subrigida*, T.S. midrib (S.E.M.), x 180.

1 E: *Anaphalis subrigida*, T.S. margin (L.M.), x 200.

1 F: *Anaphalis rupestris*, T.S. margin (S.E.M.), x 280.

Scale lines equal 50 μm.

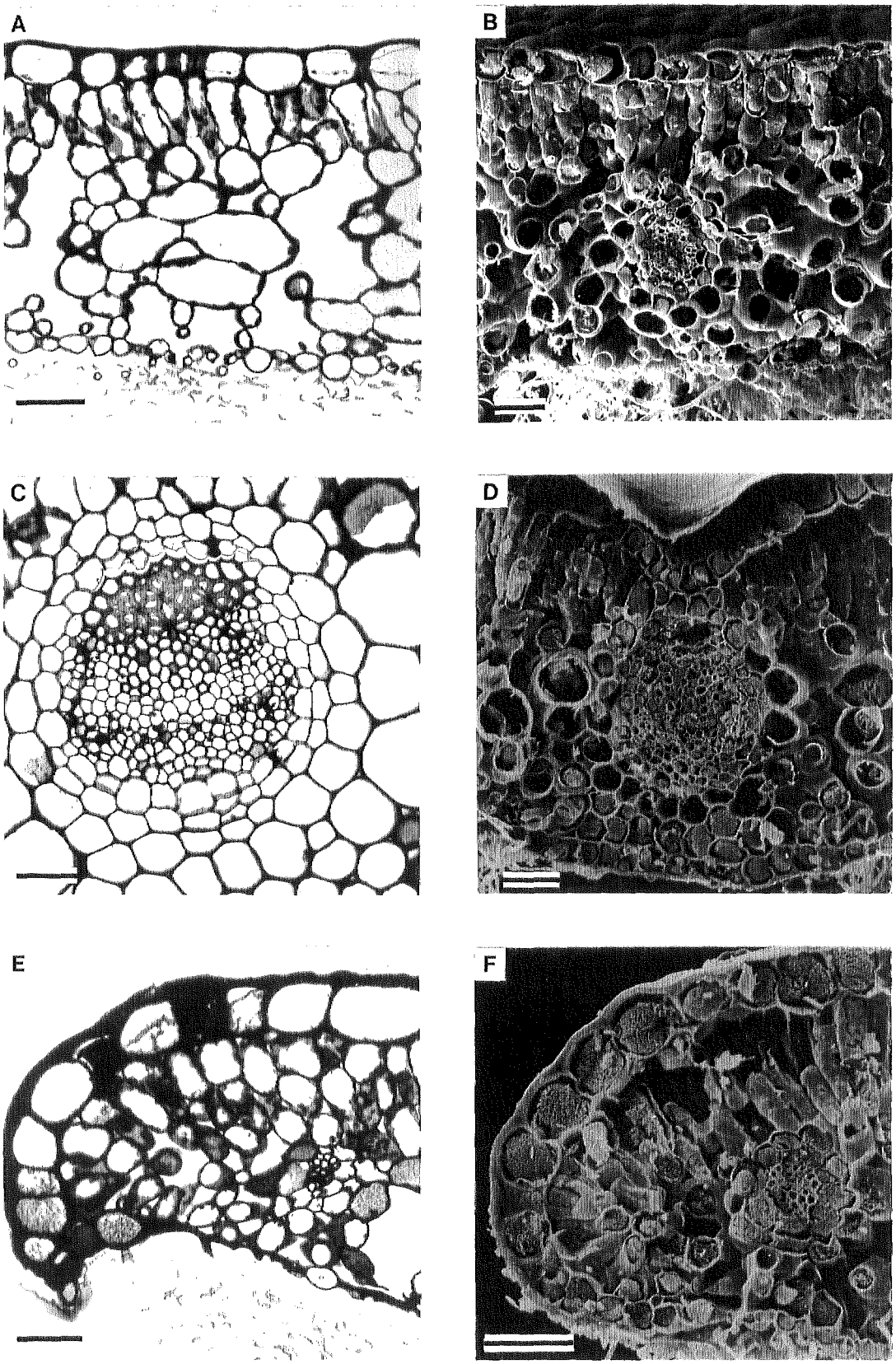

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Plate 2: Legends

2 A: *Anaphalis triplinervis*, T.S. lamina (L.M.), x 210.

2 B: *Anaphalis triplinervis*, T.S. lamina (S.E.M.), x 240.

2 C: *Anaphalis triplinervis*, T.S. midrib (L.M.), x 100.

2 D: *Anaphalis triplinervis*, T.S. margin (L.M.), x 230.

2 E: *Cassinia aculeata*, T.S. midrib (L.M.), x 200.

2 F: *Cassinia aculeata*, T.S. lamina (S.E.M.), x 120.

Scale lines equal 50 μm.

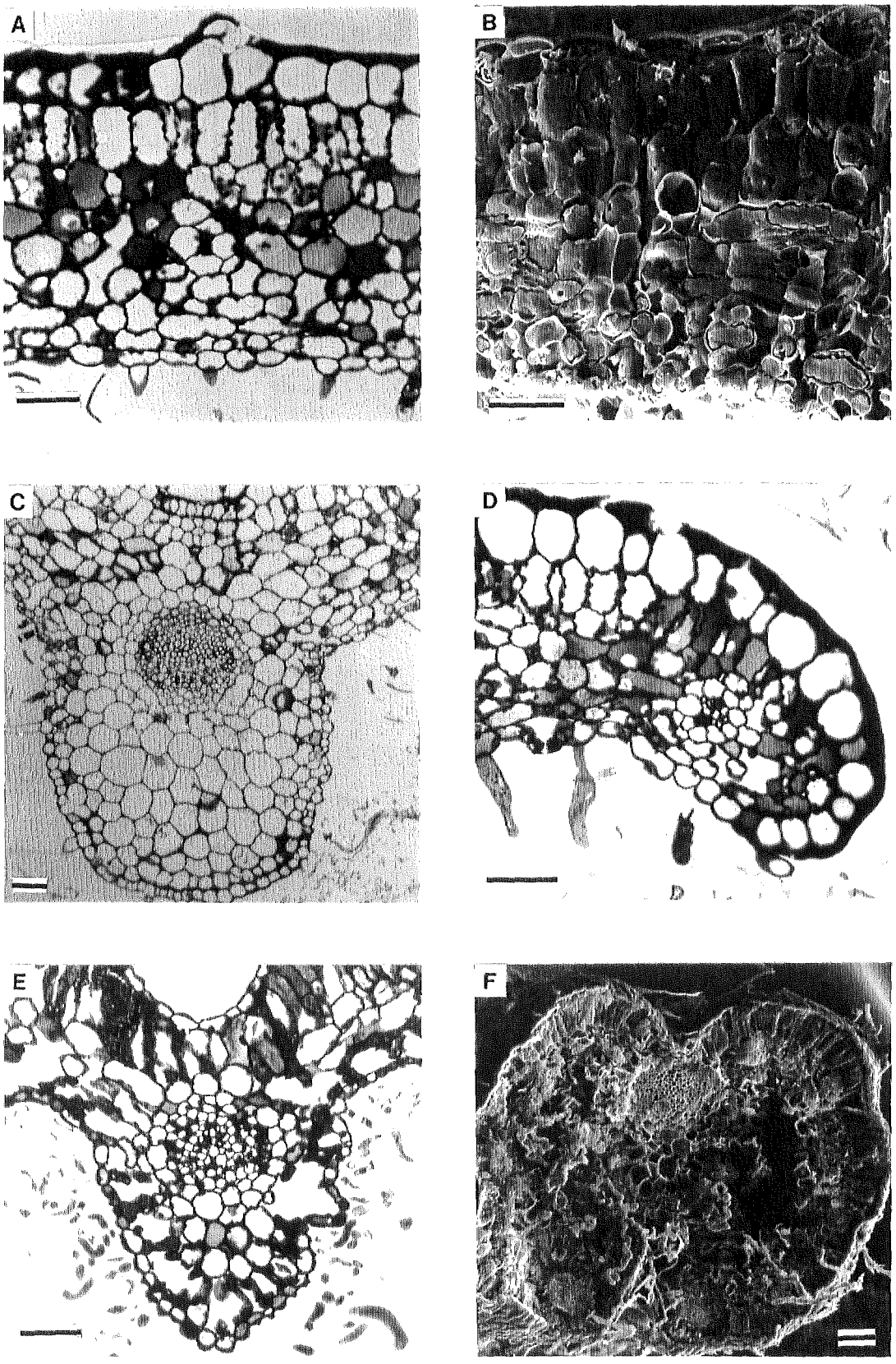
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Plate 3: Legends

3 A: *Cassinia aculeata*, T.S. lamina (L.M.), x 370.

3 B: *Cassinia aculeata*, T.S. margin (L.M.), x 370.

3 C: *Cassinia leptophylla*, T.S. midrib (L.M.), x 200.

3 D: *Cassinia fulvida*, T.S. midrib (L.M.), x 180.

3 E: *Cassinia leptophylla*, T.S. margin (L.M.), x 210.

3 F: *Cassinia fulvida*, T.S. lamina (L.M.), x 200.

Scale lines equal 50 μm.

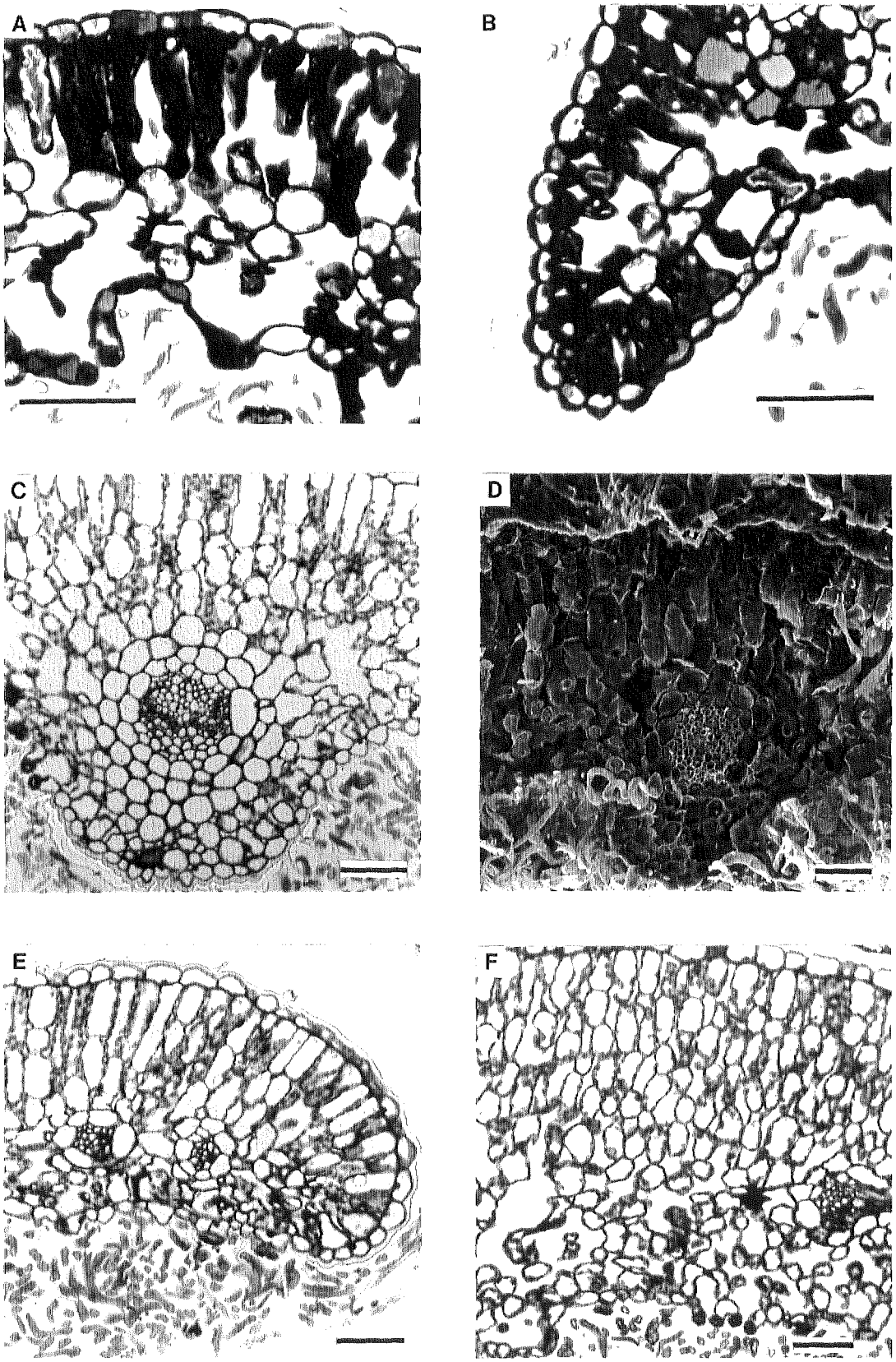
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Plate 4: Legends

4 A: *Ewartia catipes*, T.S. midrib (L.M.), x 380.

4 B: *Ewartia catipes* T.S. midrib (S.E.M.), x 340.

4 C: *Ewartia catipes*, T.S. margin (L.M.), x 380.

4 D: *Ewartia catipes*, T.S. lamina (L.M.), x 380.

4 E: *Ewartia meredithae*, T.S. midrib (L.M.), x 200.

4 F: *Ewartia meredithae*, T.S. lamina (S.E.M.), x 140.

Scale lines equal 50 μm.

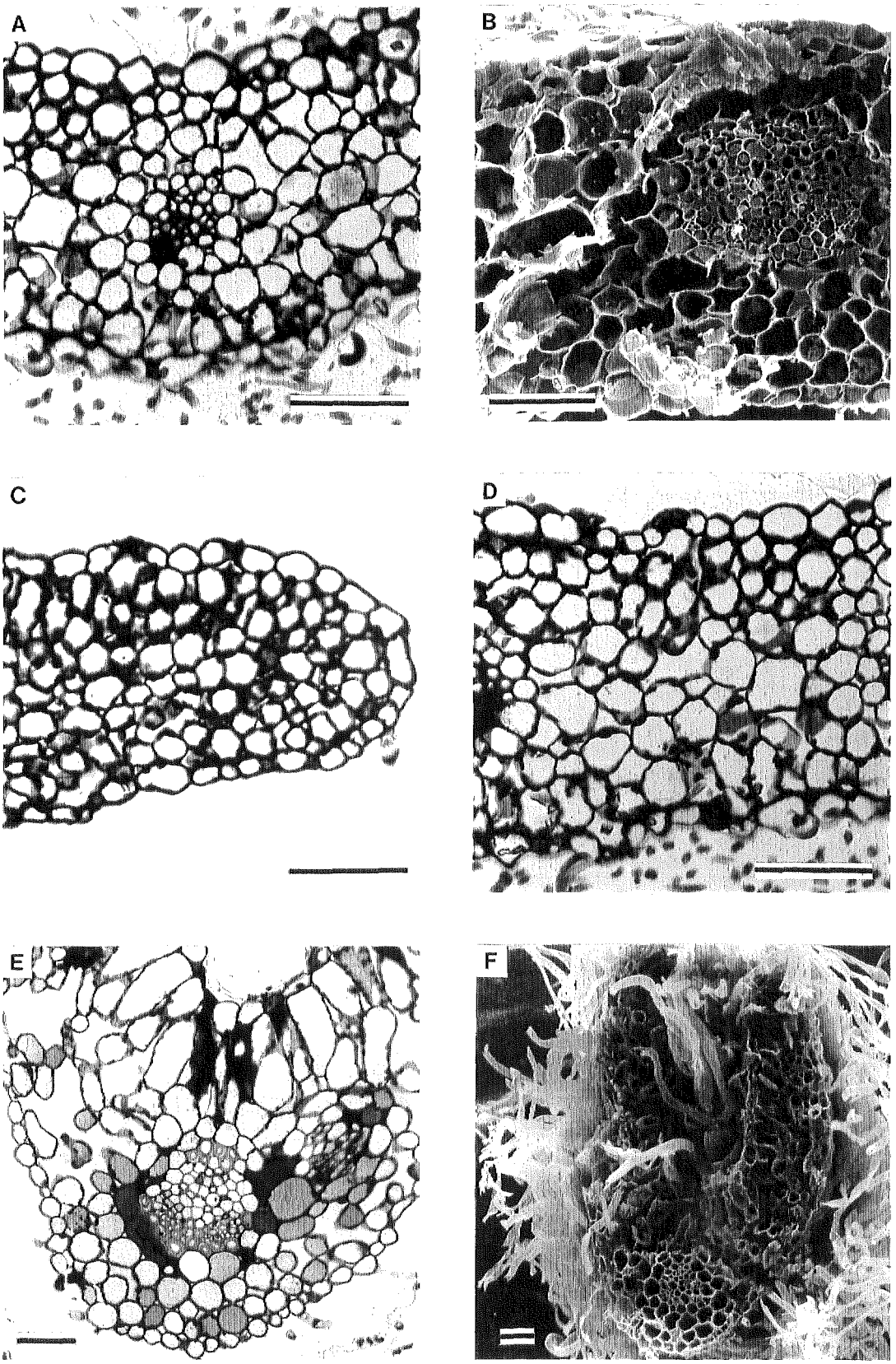
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Plate 5: Legends

5 A: *Ewartia meredithae*, T.S. lamina (L.M.), x 380.

5 B: *Ewartia meredithae*, T.S. margin (L.M.), x 250.

5 C: *Ewartia planchonii*, T.S. lamina (L.M.), x 400.

5 D: *Ewartia planchonii*, T.S. margin (L.M.), x 380.

5 E: *Ewartia sinclairii*, T.S. lamina (L.M.), x 280.

5 F: *Ewartia sinclairii*, T.S. lamina (S.E.M.), x 260.

Scale lines equal 50 μm.

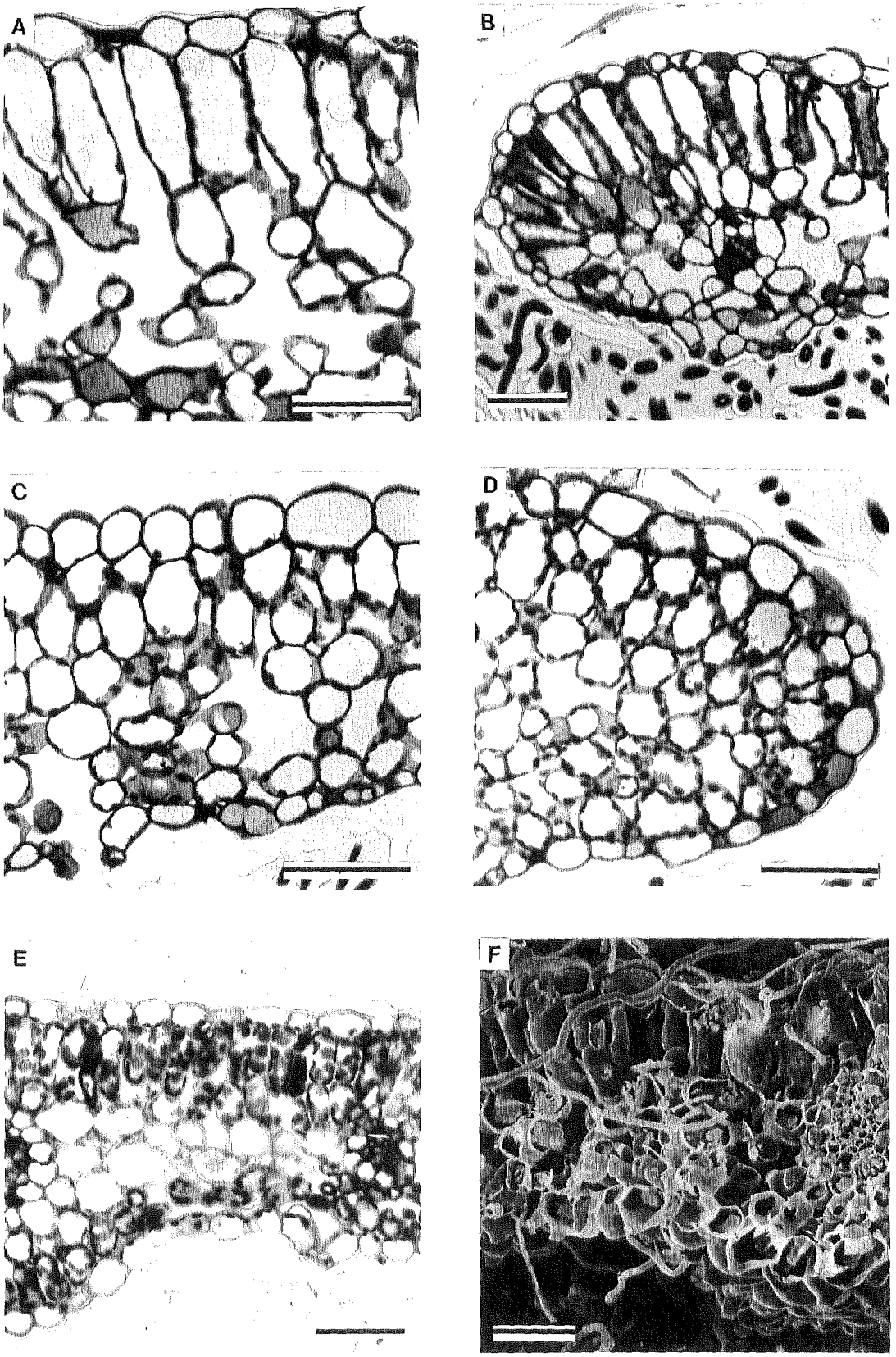
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Plate 6: Legends

6 A: *Ewartia sinclairii*, T.S. midrib (L.M.), x 200.

6 B: *Ewartia sinclairii*, T.S. midrib (S.E.M.), x 220.

6 C: *Gnaphalium involucratum*, T.S. margin (L.M.), x 390.

6 D: *Ewartia sinclairii*, T.S. margin (L.M.), x 280.

6 E: *Gnaphalium involucratum*, T.S. midrib and lamina (L.M.), x 80.

6 F: *Gnaphalium involucratum*, T.S. midrib (L.M.), x 200.

Scale lines equal 50 μm.

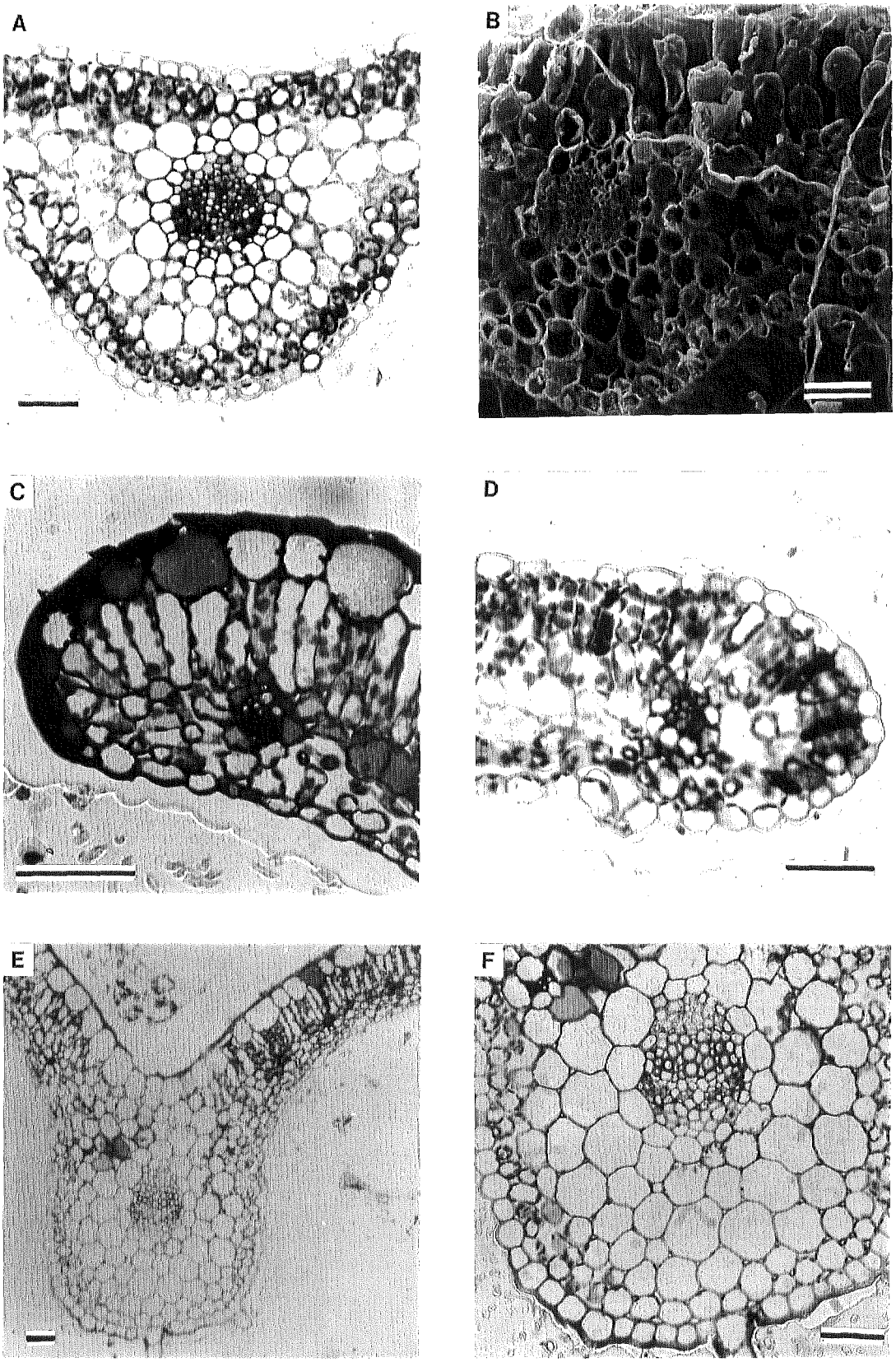
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Plate 7: Legends

7 A: *Gnaphalium mackayi*, T.S. midrib (L.M.), x 210.

7 B: *Gnaphalium mackayi*, T.S. midrib (S.E.M.), x 290.

7 C: *Gnaphalium mackayi*, T.S. lamina (L.M.), x 390.

7 D: *Gnaphalium nitidulum*, T.S. lamina (L.M.), x 390.

7 E: *Gnaphalium nitidulum*, T.S. midrib (L.M.), x 210.

7 F: *Gnaphalium nitidulum*, T.S. margin (L.M.), x 440.

Scale lines equal 50 μm.

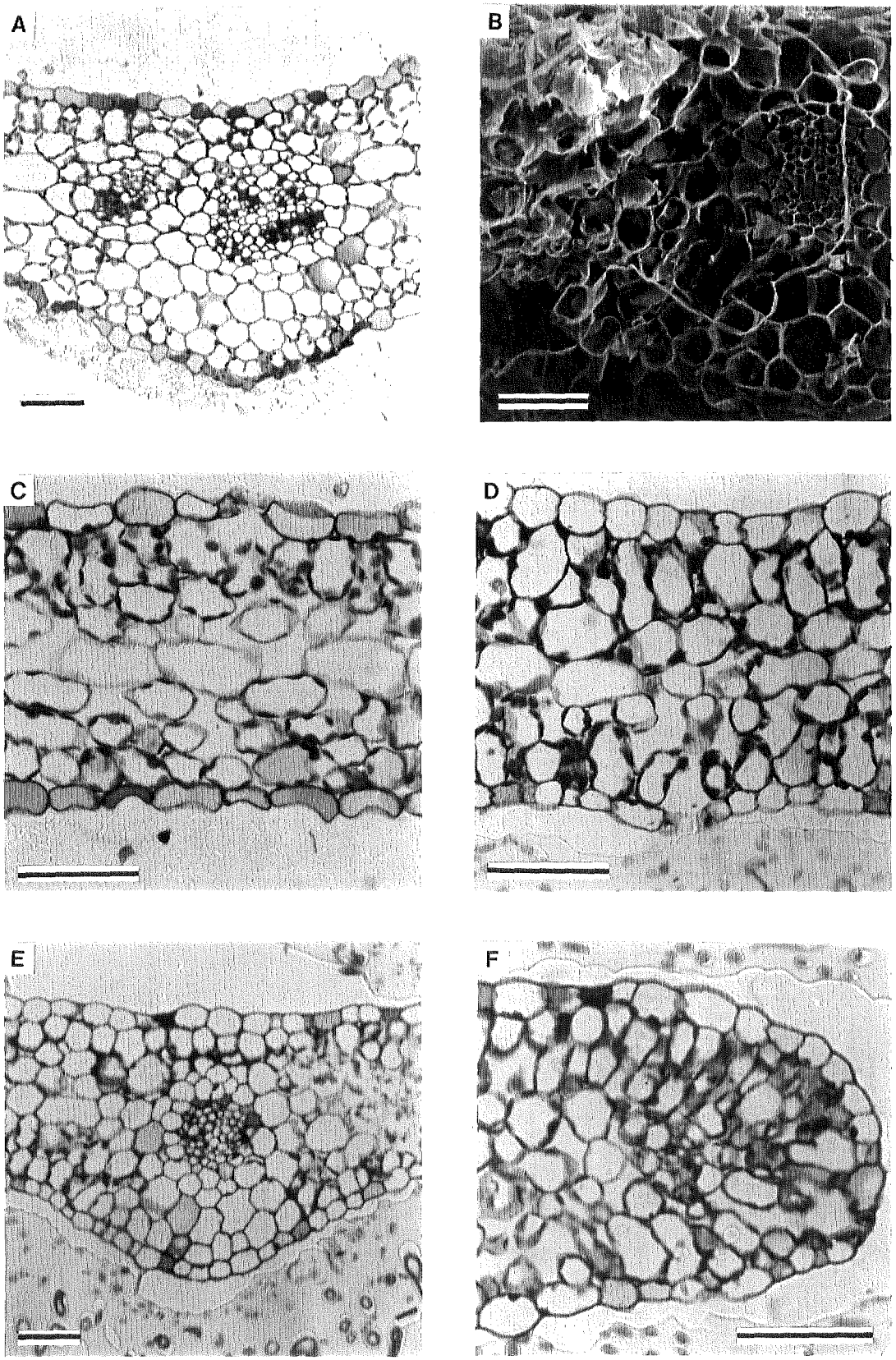
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Plate 8: Legends

8 A: *Gnaphalium traversii*, T.S. lamina (L.M.), x 390.

8 B: *Gnaphalium traversii*, T.S. midrib (L.M.), x 200.

8 C: *Haastia pulvinaris*, T.S. upper part of the lamina, lateral rib, showing secretory canal c (L.M.), x 380.

8 D: *Haastia pulvinaris*, T.S. upper part of the lamina, margin (L.M.), x 380.

8 E: *Haastia pulvinaris*, T.S. upper part of the lamina, lateral rib and lamina (L.M.), x 200.

8 F: *Haastia sinclairii*, T.S. lamina with lateral vein (L.M.), x 200.

Scale lines equal 50 μm.

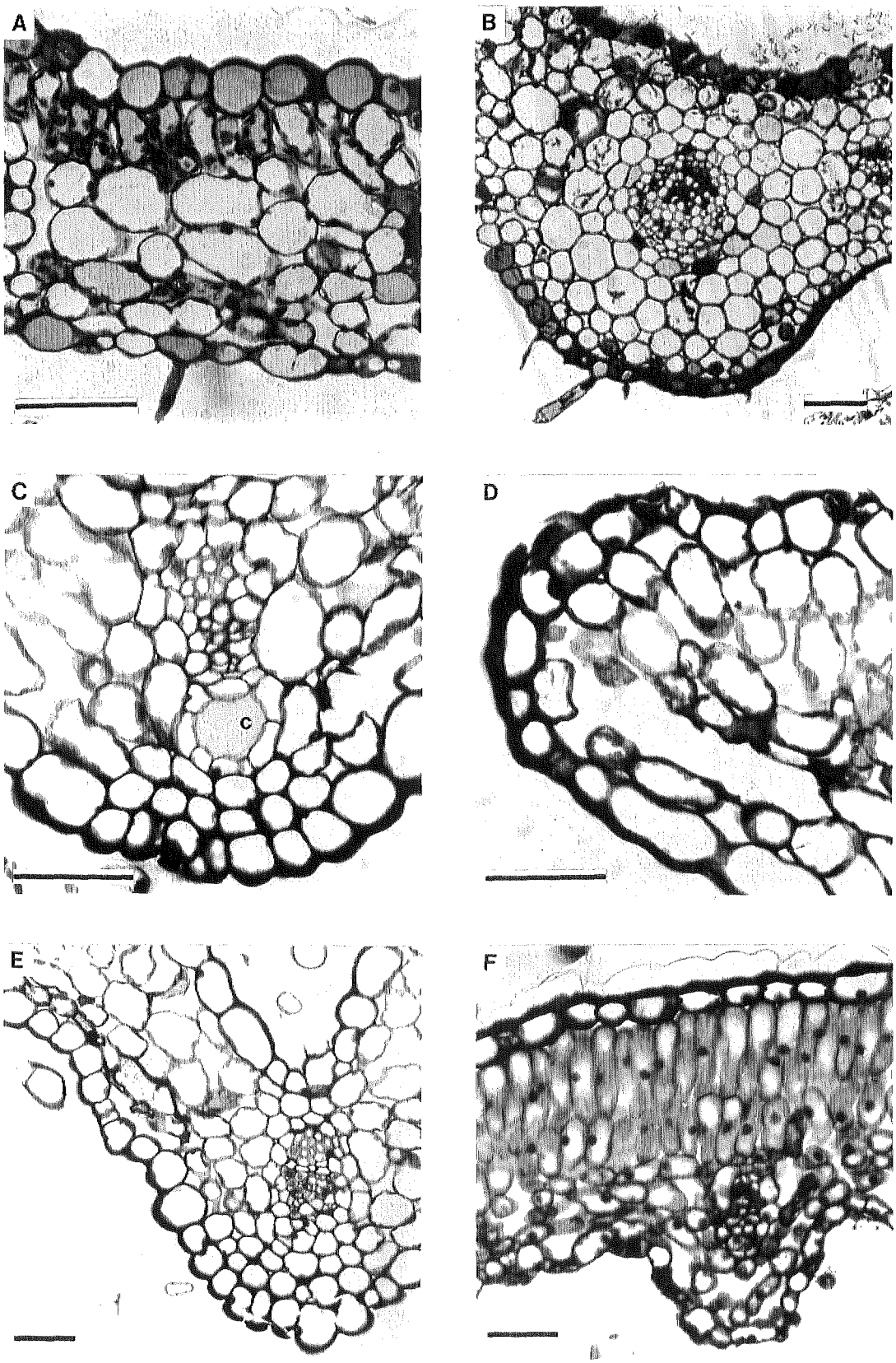
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Plate 9: Legends

9 A: *Helichrysum lanceolatum*, T.S. lamina with lateral rib showing bundle-sheath extension (L.M.), x 400.

9 B: *Helichrysum lanceolatum*, T.S. lamina (S.E.M.), x 450.

9 C: *Helichrysum bellidioides*, T.S. lamina (L.M.), x 210.

9 D: *Helichrysum bellidioides*, T.S. lamina with lateral rib (S.E.M.), x 210.

9 E: *Helichrysum bellidioides*, T.S. margin (L.M.), x 380.

9 F: *Helichrysum bellidioides*, T.S. midrib (L.M.), x 370.

Scale lines equal 50 μm.

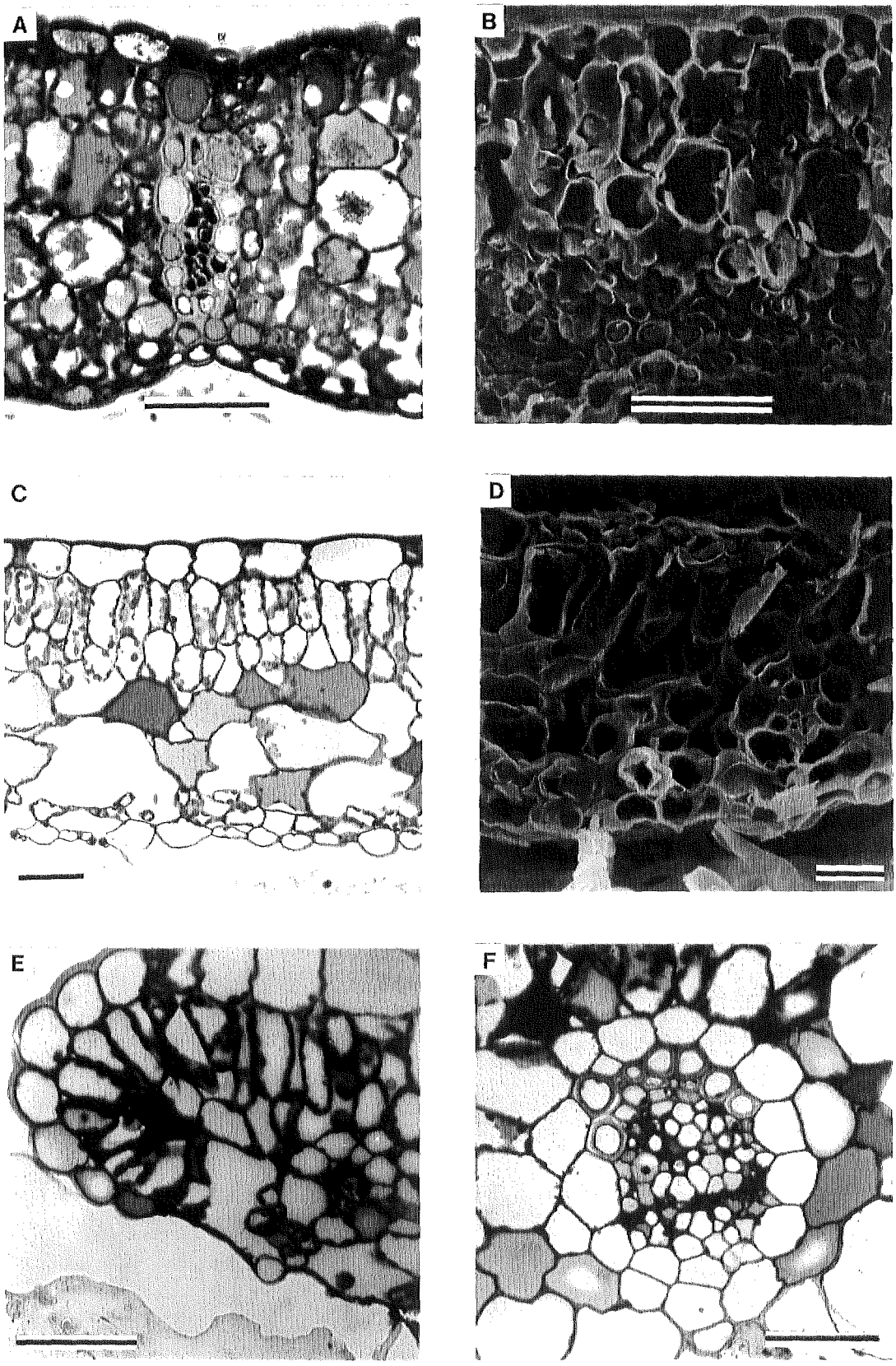
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Plate 10: Legends

10 A: *Helichrysum parvifolium*, T.S. lamina (L.M.), x 430.

10 B: *Helichrysum parvifolium*, T.S. margin (L.M.), x 480.

10 C: *Helichrysum intermedium*, T.S. midrib (L.M.), x 380.

10 D: *Helichrysum depressum*, T.S. midrib (L.M.), x 380.

10 E: *Helichrysum depressum*, T.S. lamina (L.M.), x 400.

10 F: *Helichrysum depressum*, T.S. margin (L.M.), x 400.

Scale lines equal 50 μm.

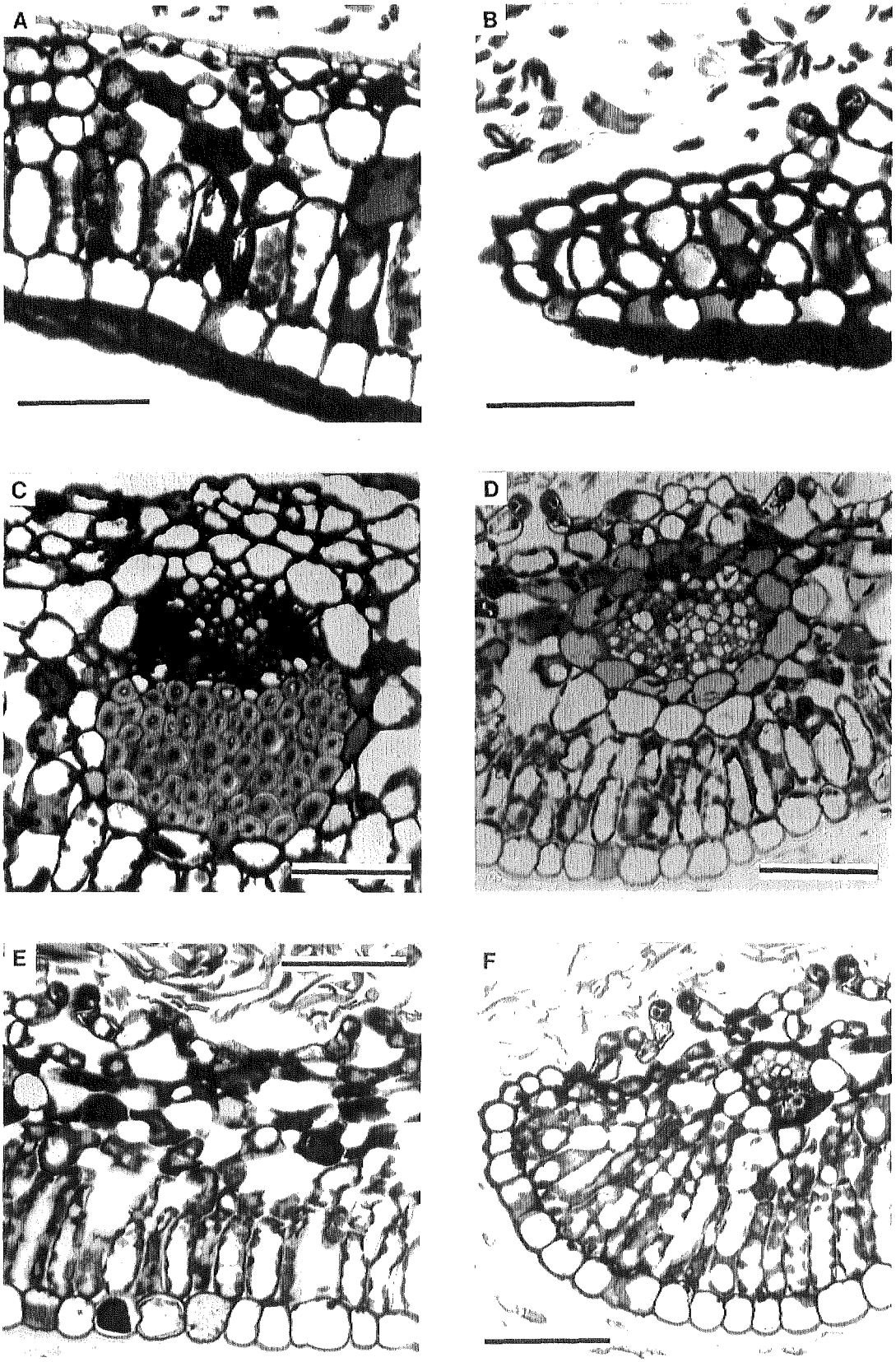
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Plate 11: Legends

11 A: *Helichrysum depressum*, T.S. base of lamina showing sclerenchyma (L.M.), x 380.

11 B: *Helichrysum depressum*, T.S. base of lamina showing sclerenchyma (S.E.M.), x 680.

11 C: *Helichrysum dimorphum*, T.S. lamina of the scale-like leaf (L.M.), x 380.

11 D: *Helichrysum dimorphum*, T.S. lamina of the normal leaf (S.E.M.), x 560.

11 E: *Helichrysum filicaule*, T.S. midrib (L.M.), x 200.

11 F: *Helichrysum filicaule*, T.S. lamina with lateral rib (S.E.M.), x 450.

Scale lines equal 50 μm.

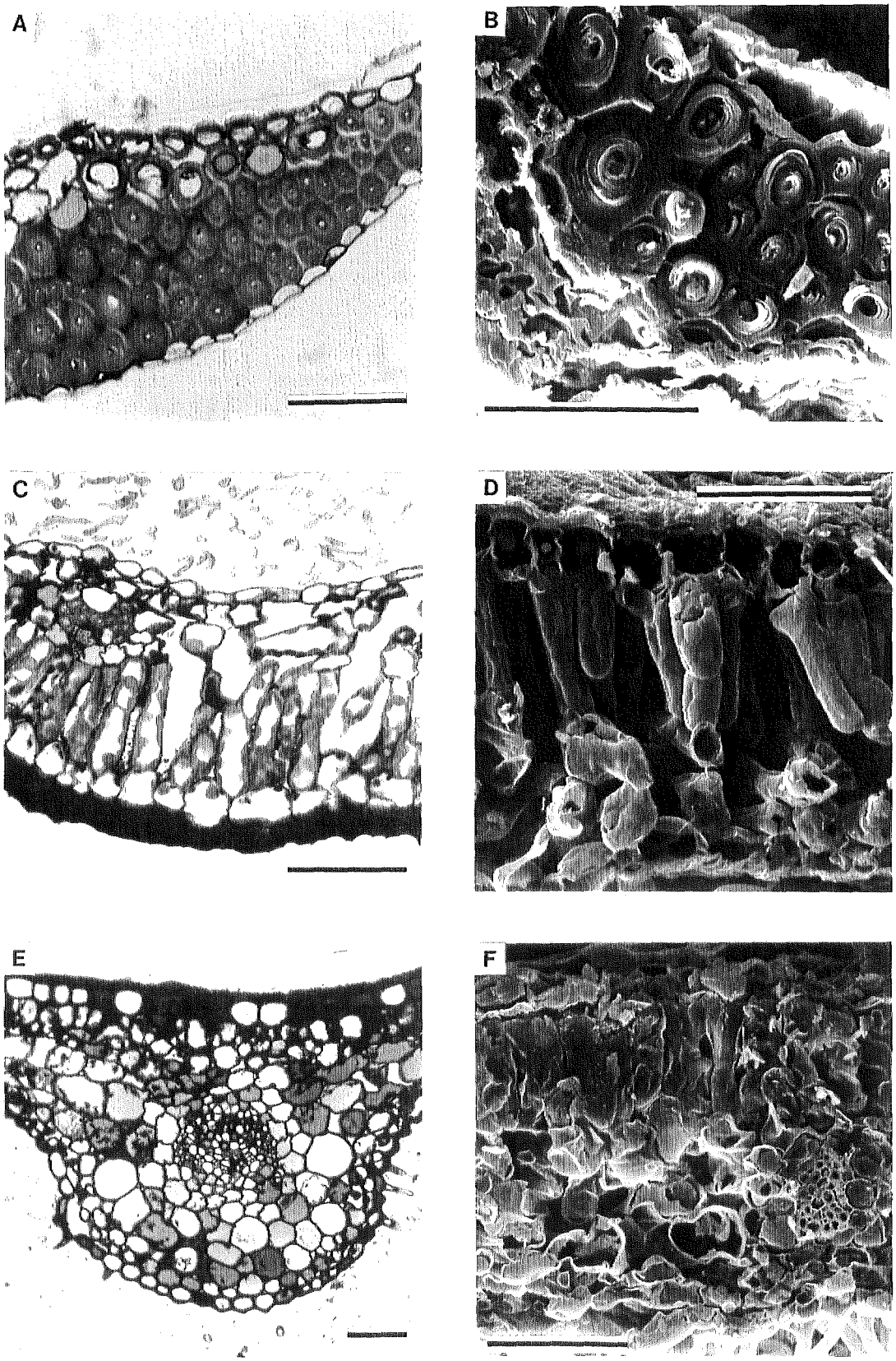
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Plate 12: Legends

12 A: *Helichrysum obcordatum*, T.S. lamina (L.M.), x 390.

12 B: *Helichrysum obcordatum*, T.S. margin (L.M.), x 380.

12 C: *Leucogenes grandiceps*, T.S. lamina (L.M.), x 380.

12 D: *Leucogenes grandiceps*, T.S. midrib (L.M.), x 380.

12 E: *Leucogenes grandiceps*, T.S. margin (L.M.), x 250.

12 F: *Leucogenes leontopodium*, T.S. midrib (L.M.), x 380.

Scale lines equal 50 μm.

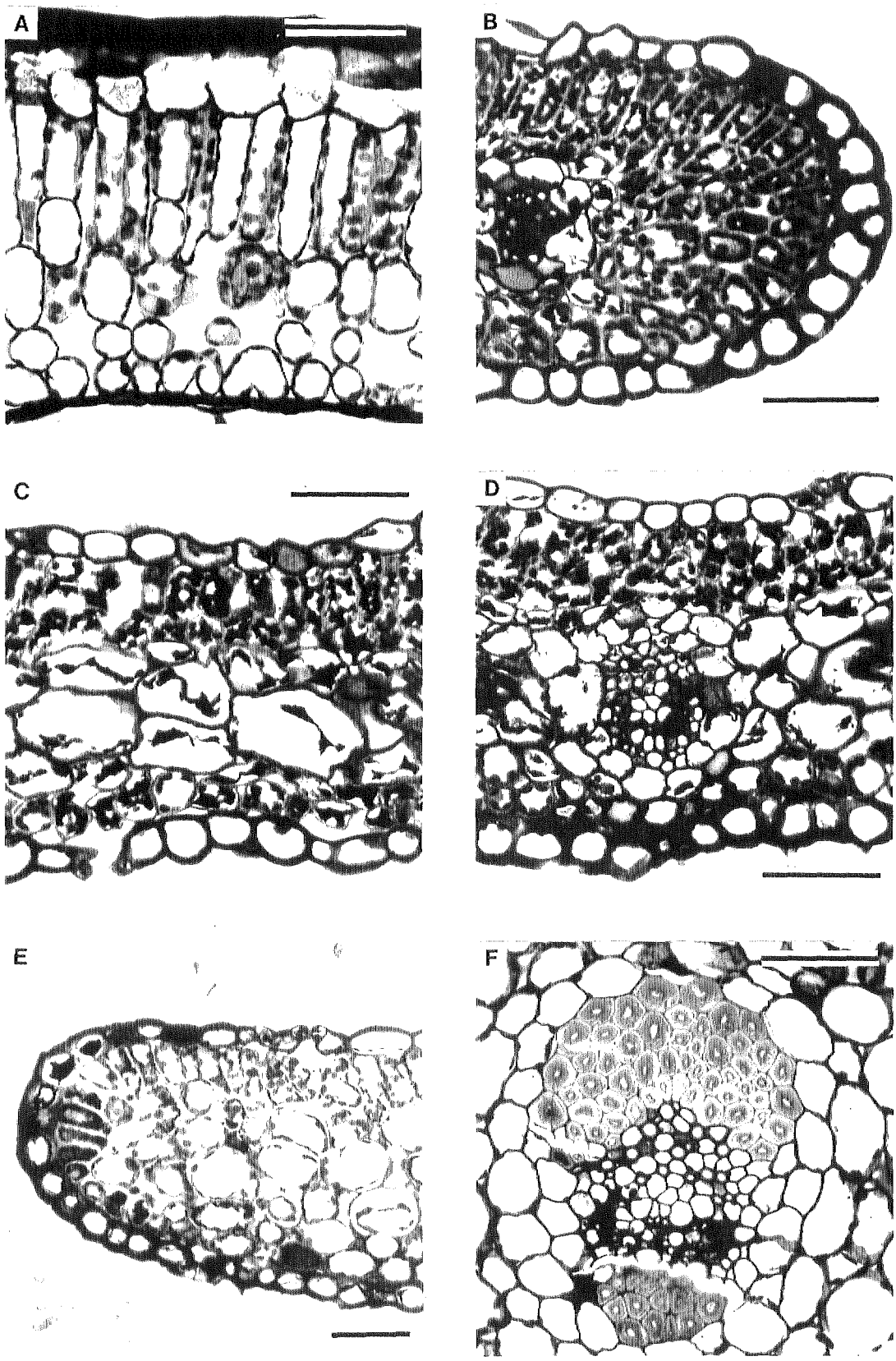
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Plate 13: Legends

13 A: *Leucogenes* "Peel", T.S. lamina (L.M.), x 230.

13 B: *Leucogenes leontopodium*, T.S. lamina (S.E.M.), x 190.

13 C: *Pseudognaphalium luteoalbum*, T.S. midrib (L.M.), x 200.

13 D: *Pseudognaphalium luteoalbum*, T.S. midrib (S.E.M.), x 90.

13 E: *Pseudognaphalium luteoalbum*, T.S. lamina (L.M.), x 370.

13 F: *Pseudognaphalium luteoalbum*, T.S. margin (L.M.), x 370.

Scale lines equal 50 μm.

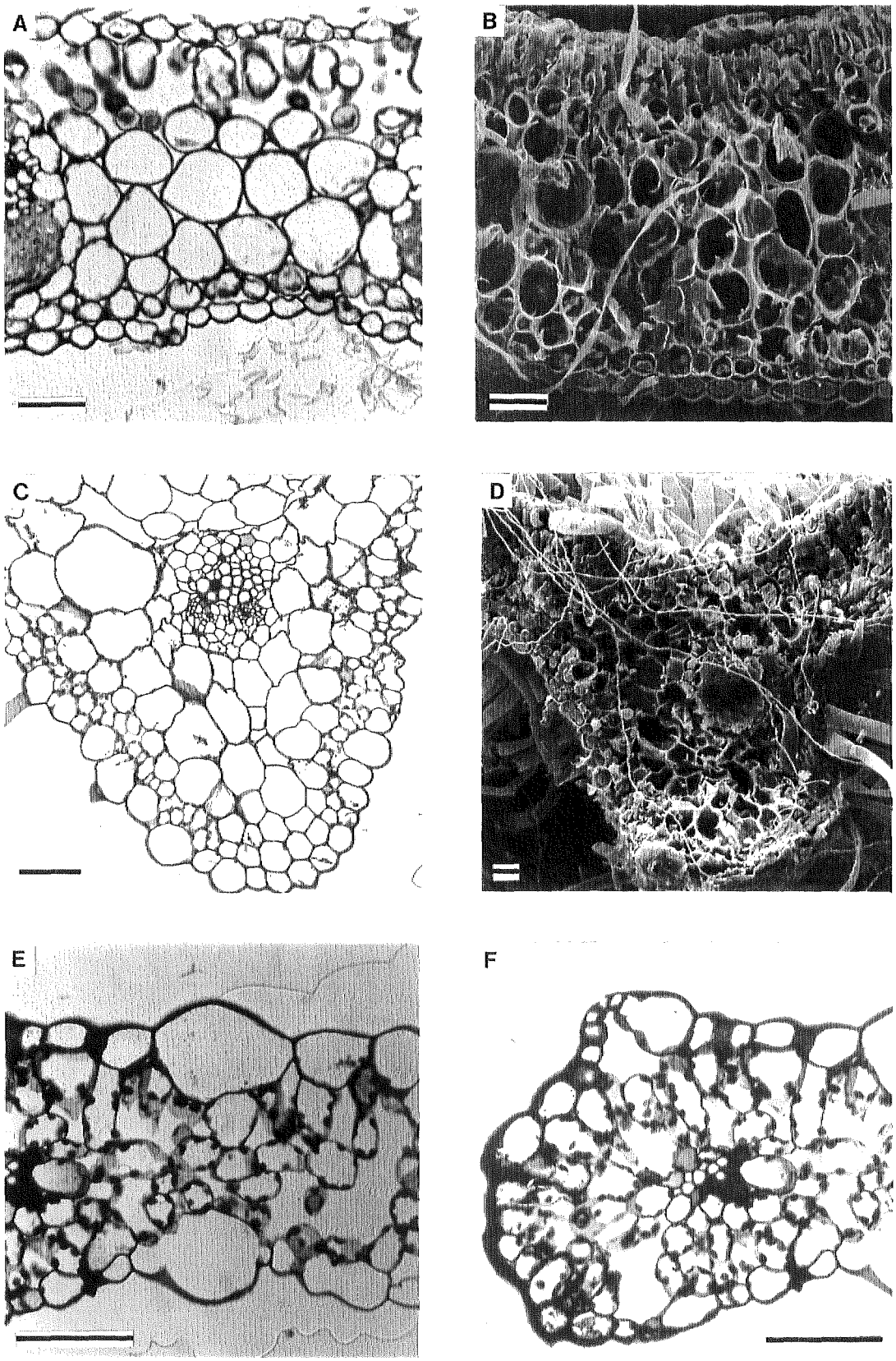
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Plate 14: Legends

14 A: *Pterygopappus lawrencii*, T.S. midrib (L.M.), x 260.

14 B: *Pterygopappus lawrencii*, T.S. lamina (L.M.), x 260.

14 C: *Raoulia bryoides*, T.S. margin (L.M.), x 380.

14 D: *Raoulia bryoides*, T.S. lamina (L.M.), x 380.

14 E: *Raoulia bryoides*, T.S. midrib (L.M.), x 380.

14 F: *Raoulia eximia*, T.S. midrib (L.M.), x 380.

Scale lines equal 50 μm.

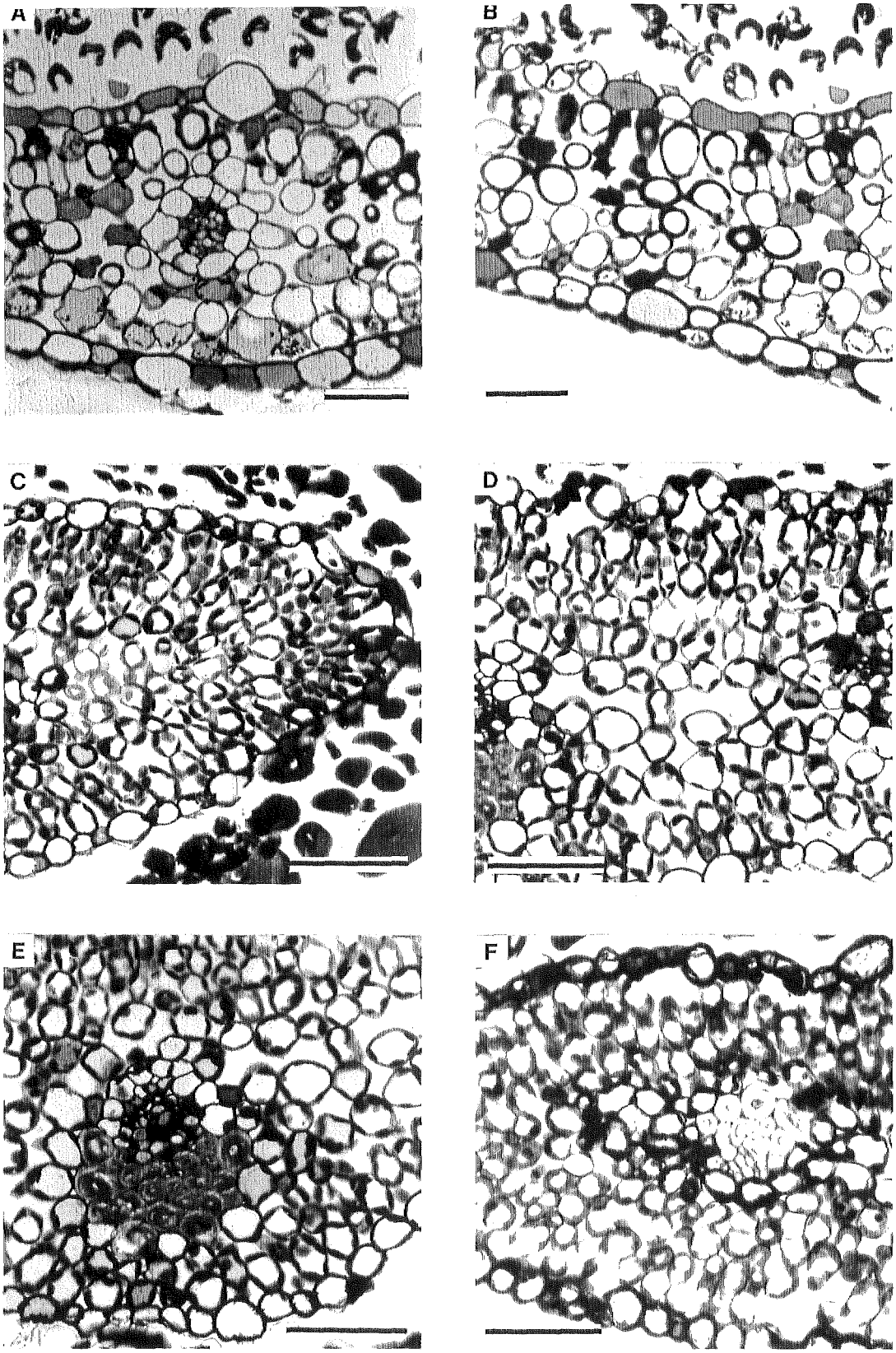
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Plate 15: Legends

15 A: *Raoulia cinerea*, T.S. midrib (L.M.), x 200.

15 B: *Raoulia cinerea*, T.S. margin (L.M.), x 180.

15 C: *Raoulia cinerea*, T.S. lamina (L.M.), x 420.

15 D: *Raoulia cinerea*, T.S. lamina (S.E.M.), x 300.

15 E: *Raoulia grandiflora*, T.S. midrib (L.M.), x 220.

15 F: *Raoulia grandiflora*, T.S. midrib (S.E.M.), x 280.

Scale lines equal 50 μm.

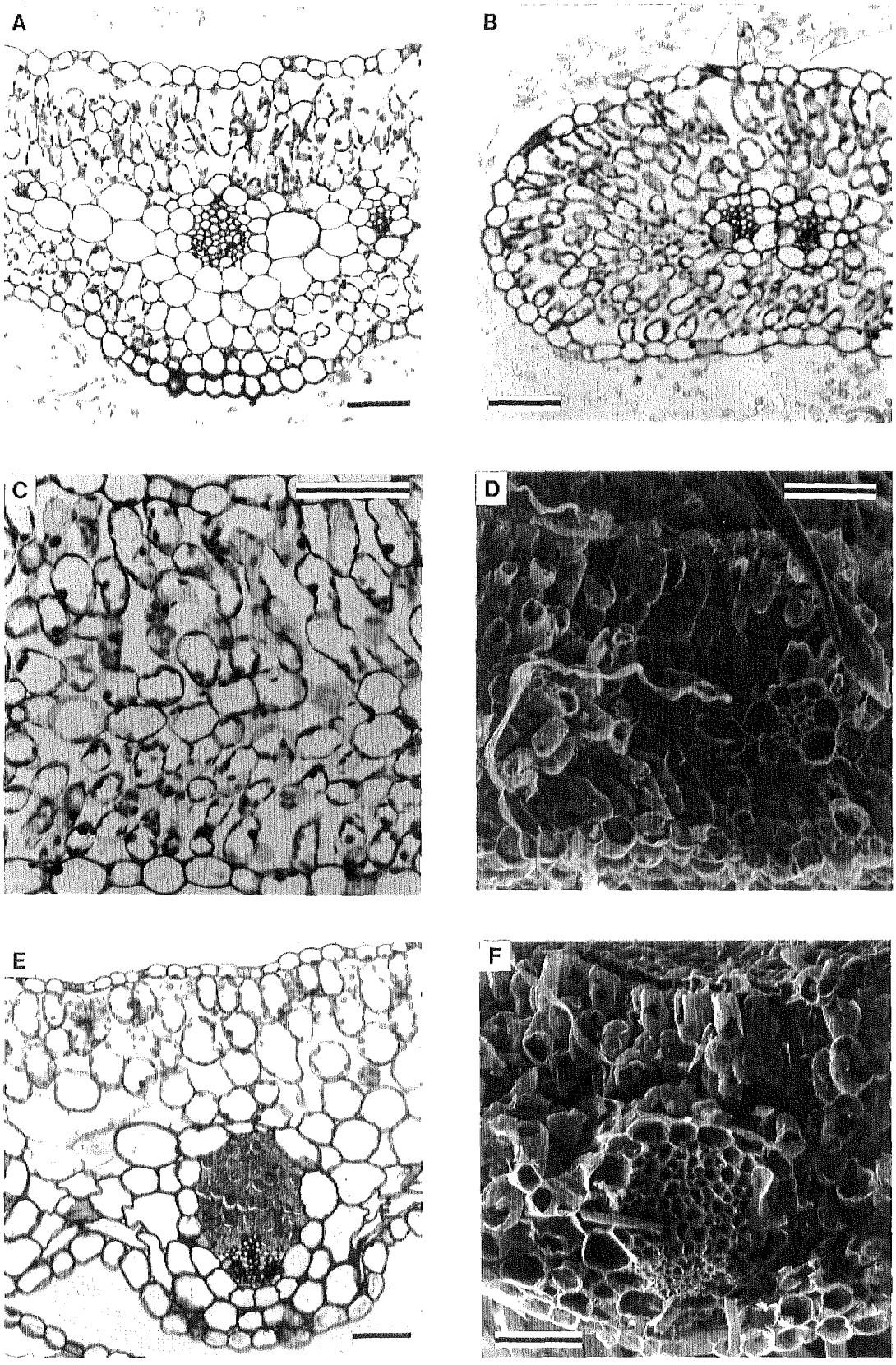
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Plate 16: Legends

16 A: *Raoulia grandiflora*, T.S. lamina (L.M.), x 200.

16 B: *Raoulia grandiflora*, T.S. margin (L.M.), x 210.

16 C: *Raoulia glabra*, T.S. midrib (L.M.), x 210.

16 D: *Raoulia glabra*, T.S. midrib (S.E.M.), x 300.

16 E: *Raoulia tenuicaulis*, T.S. midrib (L.M.), x 380.

16 F: *Raoulia hookeri*, T.S. midrib (S.E.M.), x 350.

Scale lines equal 50 μm.

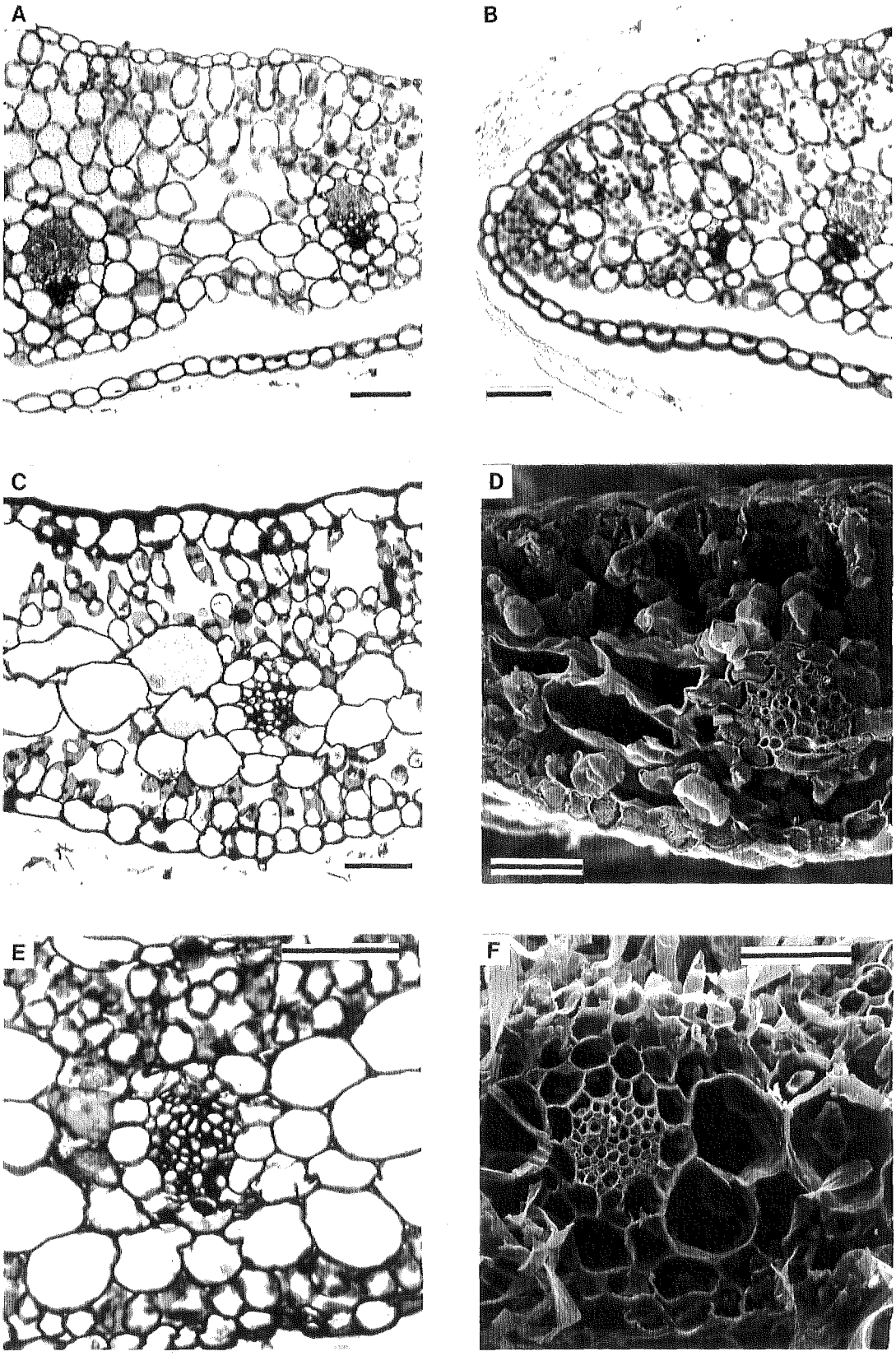
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Plate 17: Legends

17 A: *Raoulia tenuicaulis*, T.S. lamina (L.M.), x 390.

17 B: *Raoulia tenuicaulis*, T.S. lamina (S.E.M.), x 370.

17 C: *Raoulia* "M", T.S. midrib (L.M.), x 210.

17 D: *Raoulia hookeri*, T.S. margin (L.M.), x 420.

17 E: *Raoulia* "M", T.S. lamina (L.M.), x 360.

17 F: *Raoulia* "M", T.S. margin (L.M.), x 360.

Scale lines equal 50 μm.

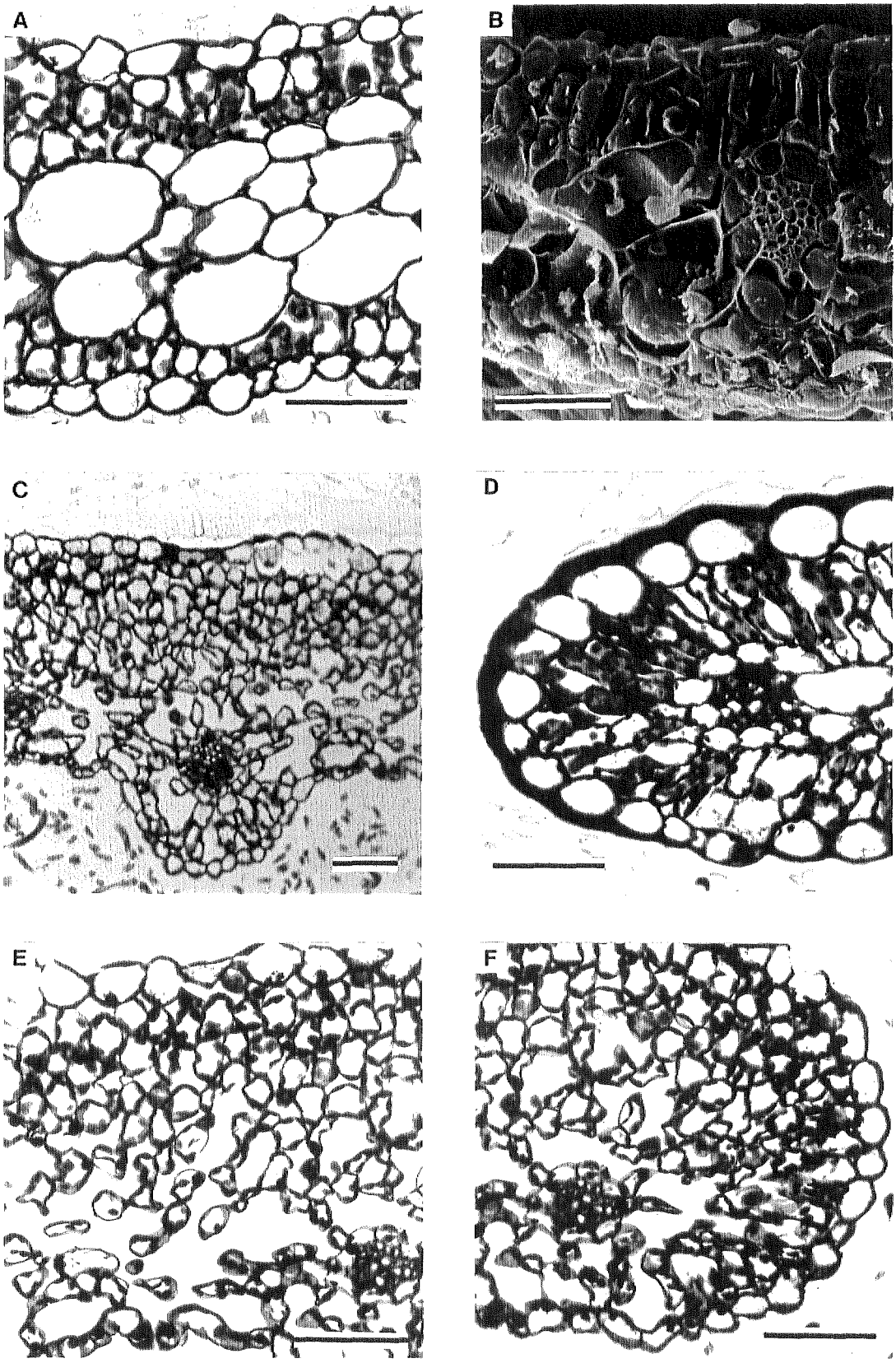
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Plate 18: Legends

18 A: *Raoulia petriensis*, T.S. midrib (L.M.), x 360.

18 B: *Raoulia petriensis,* T.S. lamina (L.M.), x 360.

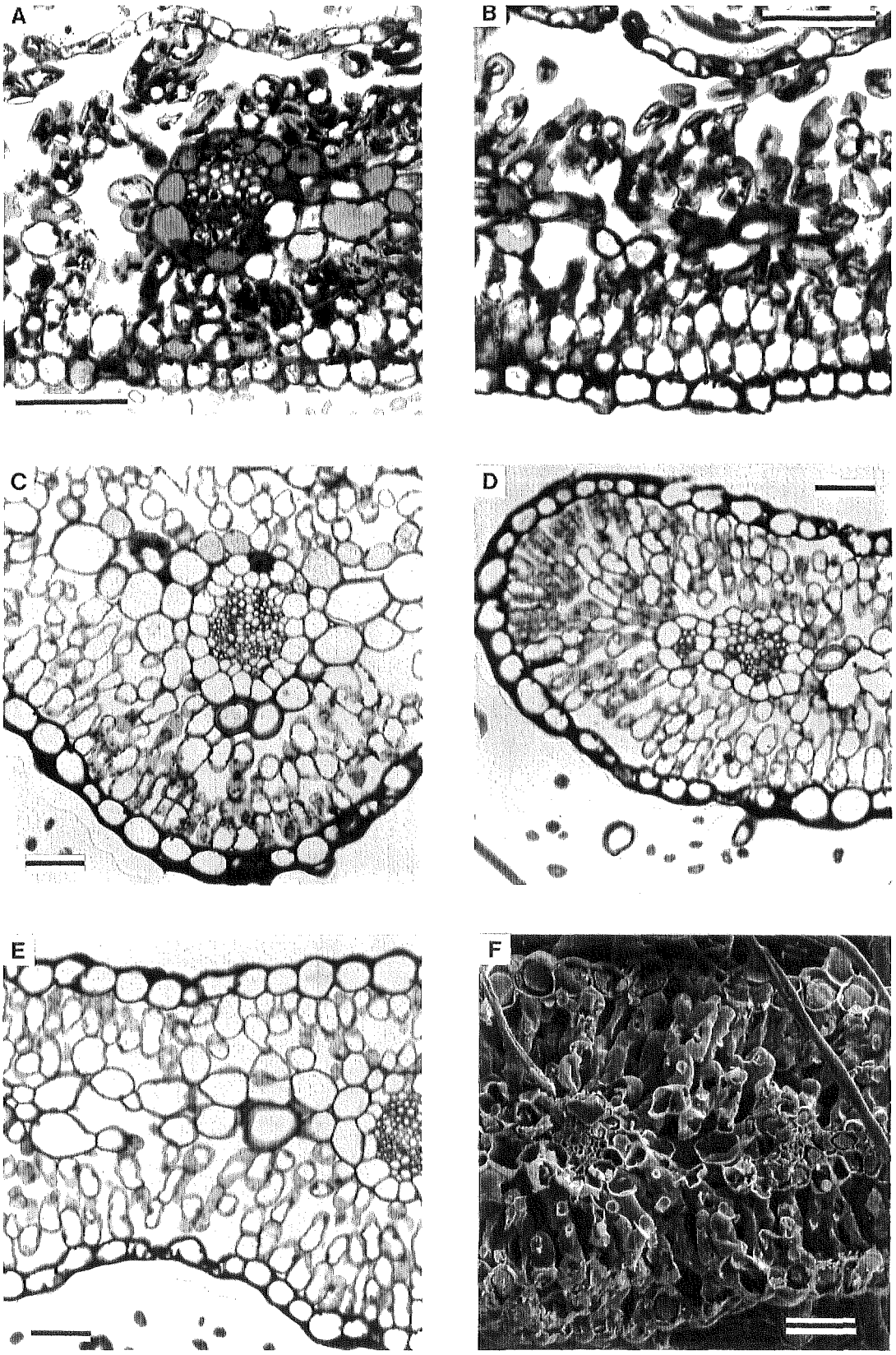
18 C: Genus "Z", T.S. midrib (L.M.), x 200.

18 D: Genus "Z", T.S. margin (L.M.), x 200.

18 E: Genus "Z", T.S. lamina (L.M.), x 200.

18 F: Genus "Z", T.S. lamina (S.E.M.), x 220.

Scale lines equal 50 μm.


A
B
C
D
E
F

2.4.3. Numerical analyses

The characters used for the phenetic analyses are listed in Appendix 2. 10 variables are continuous and 39 discrete non-ordered characters.

The phenogram formed by using UPGMA is shown in Figure 2.2. The most evident features are the following:

Of the 8 genera represented by more than one species only two, *Haastia* and *Leucogenes*, form a discrete cluster. There are four major clusters, a, b, c, d and one species, *Helichrysum filicaule*, which is very isolated.

Cluster a has two very distinct components, the first comprising the New Zealand species of *Anaphalis*, which link at very high similarity levels (more than 0.94), and the second including *Gnaphalium involucratum*, *G. umbricola* and *Anaphalis triplinervis*. The last mentioned species joins the two species of *Gnaphalium* at the similarity level of 0.89.

Cluster b is formed by the two species of *Haastia* uniting with *Helichrysum lanceolatum* at very low similarity levels (0.77). Cluster b unites with cluster a at 0.72 and this combined cluster is clearly separated from all the other OTUs.

Cluster c includes the inverse-dorsiventral species of *Helichrysum*. *Helichrysum coralloides*, *H. intermedium* and *H. parvifolium* are more similar to one another than to *H. depressum* and *H. dimorphum* (scale-like leaf).

The remaining species are combined in cluster d, which has two major constituents e and f as well as the isolated Genus "Z".

Within cluster e, *Pseudognaphalium luteoalbum* has an isolated position. *Helichrysum bellidioides* and *Raoulia* "M", clustering at the relatively low similarity level of 0.87, join the other members of cluster e at 0.82. These other members form two distinct clusters. One of them consists of the two Tasmanian species of *Cassinia*, the second of *Helichrysum dimorphum* (normal leaf), the New Zealand species of *Cassinia*, the Tasmanian species of *Helichrysum* and two of the Tasmanian species of *Ewartia* (*E. meredithae* and *E. planchonii*), but not the third (*E. catipes*). *Cassinia fulvida* and *H. backhousii* are joined at 1.00 and join *C. leptophylla* at the

	OTU	Phenogram linkage levels
43	R.hectori	1.0000
45	R."L"	1.0000
38	R.bryoides	1.0000
40	R.eximia	0.9324
10	E.catipes	0.8645
44	R.hookeri	0.9318
48	R.tenuicaulis	0.8956
41	R.glabra	0.8394
42	R.grandiflora	0.8210
37	Pterygopappus	0.8444
47	R.petriensis	0.8078
33	L.leontopodium	0.9946
35	L."Peel"	0.9687
34	L."Marlborough"	0.9172
32	L.grandiceps	0.7995
39	R.cinerea	0.7758
15	G.mackayi	0.9396
16	G.nitidulum	0.8900
17	G.traversii	0.8513
13	E.sinclairii	0.7572
49	Genus"Z"	0.7561
22	He.bellidioides	0.8712
46	R."M"	0.8270
6	C.aculeata	0.9066
9	C.longifolia	0.8589
7	C.fulvida	1.0000
21	He.backhousii	0.9745
8	C.leptophylla	0.8907
11	E.meredithae	0.9421
12	E.planchonii	0.8942
26	He.dimorphumn	0.9143
30	He.obcordatum	0.7873
36	Pseudognaphalium	0.7117
23	He.coralloides	1.0000
31	He.parvifolium	0.9826
28	He.intermedium	0.9254
24	He.depressum	0.9592
25	He.dimorphums	0.6547
19	Ha.pulvinaris	0.9102
20	Ha.sinclairii	0.7731
29	He.lanceolatum	0.7205
1	A.keriensis	0.9668
4	A.trinervis	0.9546
3	A.subrigida	0.9425
2	A.rupestris	0.7622
14	G.involucratum	0.9623
18	G.umbricola	0.8914
5	A.triplinervis	0.6565
27	He.filicaule	0.0000

Cophenetic correlation = 0.812

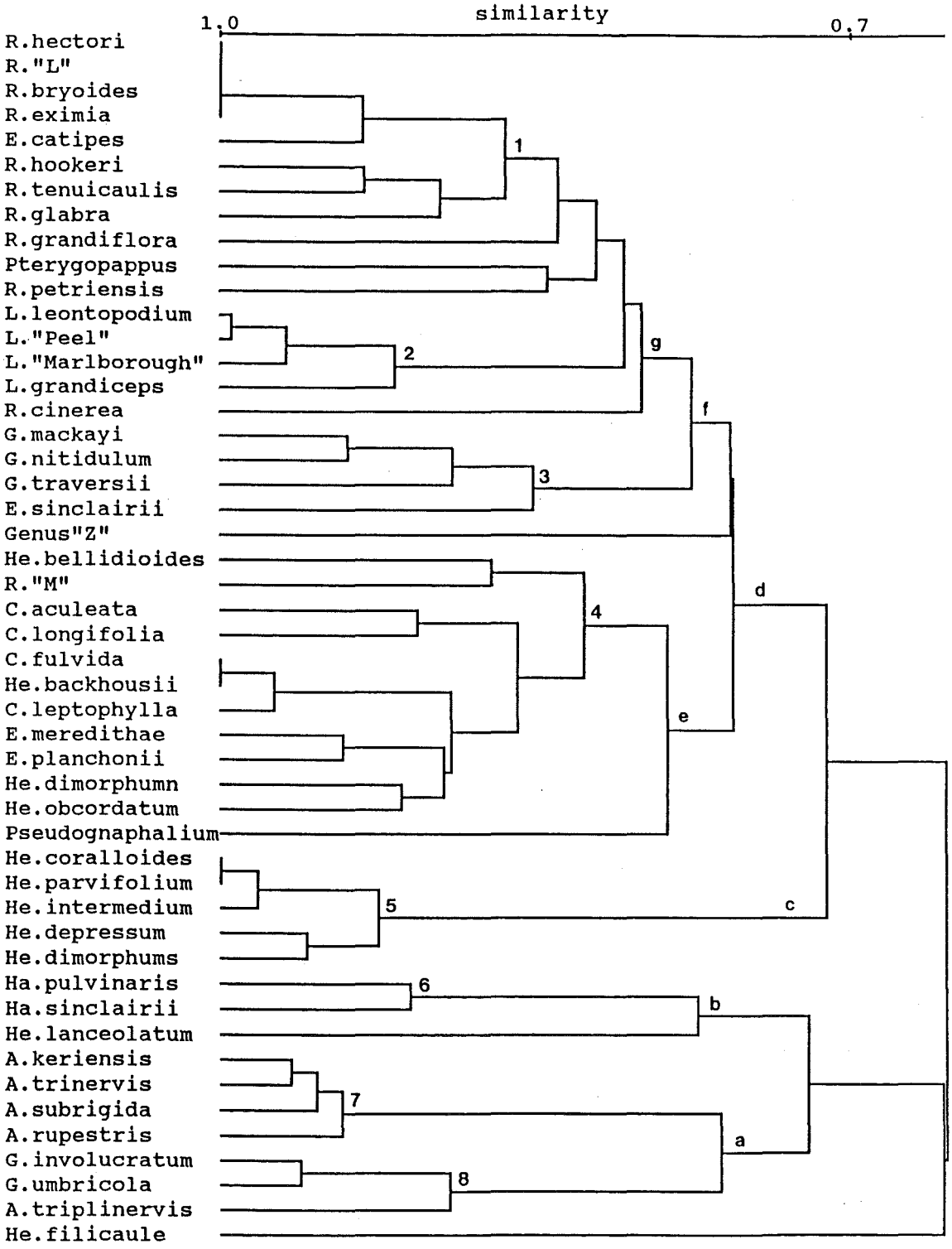


Figure 2.2. UPGMA phenogram from anatomical data with Gower's coefficient.

very high similarity level of 0.97. *E. meredithae* and *E. planchonii*, united at 0.94, and *H. dimorphum* (normal leaf) and *H. obcordatum*, united at 0.91, join at 0.89.

Cluster f contains the remaining species. A cluster is formed by *Gnaphalium mackayi, G. nitidulum, G. traversii* and *Ewartia sinclairii*. They are united at 0.85 and join cluster g at the similarity level of 0.77.

Cluster g consists of three components. One component includes all *Leucogenes* species, the second component is the isolated *Raoulia cinerea* and the third component is formed by *Pterygopappus lawrencii, Ewartia catipes* and the remaining species of *Raoulia*. Within this third component, *R. petriensis, Pterygopappus lawrencii* and *R. grandiflora* have isolated positions. *R. hectori, R.* "L", *R. bryoides* and *R. eximia* are identical and join *E. catipes* at 0.93, while *R. hookeri, R. tenuicaulis* and *R. glabra* are united at 0.89, with both clusters combined at 0.86.

The cophenetic correlation coefficient has the value of 0.812. The similarity matrix is given in Appendix 6.

The phenogram formed by using single linkage clustering is shown in Figure 2.3. The most evident features are the following:

The most isolated taxon is *Helichrysum filicaule*. The New Zealand species of *Anaphalis* form a cluster on their own. They are combined at the very high similarity level of 0.94. *Helichrysum lanceolatum*, Genus "Z", *Pseudognaphalium luteoalbum* and *Raoulia cinerea* each has only slight similarity to any other species. A few quite isolated clusters can be distinguished. *Gnaphalium involucratum* and *G. umbricola* are united with *Anaphalis triplinervis* at 0.9. The two species of *Haastia* pair at 0.91. The inverse-dorsiventral species of *Helichrysum* and also the species of *Leucogenes* join at 0.92. All the other species are associated in the two clusters a and b. *Gnaphalium mackayi, G. nitidulum* and *G. traversii* cluster at 0.89 and are joined by the isolated *Ewartia sinclairii* and the other species of cluster a at 0.86. These form four clusters. The cluster of *Cassinia fulvida* and *Helichrysum backhousii* with *C. leptophylla* (at 0.97) associates with the paired *Ewartia meredithae* and *E. planchonii* and the remotely paired

	OTU	Phenogram linkage levels
43	R.hectori	1.0000
45	R."L"	1.0000
40	R.eximia	1.0000
38	R.bryoides	0.9475
10	E.catipes	0.9146
44	R.hookeri	0.9318
48	R.tenuicaulis	0.9305
41	R.glabra	0.8851
42	R.grandiflora	0.8632
47	R.petriensis	0.8531
37	Pterygopappus	0.8515
26	He.dimorphumn	0.9143
30	He.obcordatum	0.9076
7	C.fulvida	1.0000
21	He.backhousii	0.9745
8	C.leptophylla	0.9091
11	E.meredithae	0.9421
12	E.planchonii	0.8987
6	C.aculeata	0.9066
9	C.longifolia	0.8837
22	He.bellidioides	0.8712
46	R."M"	0.8560
15	G.mackayi	0.9396
16	G.nitidulum	0.8918
17	G.traversii	0.8567
13	E.sinclairii	0.8473
33	L.leontopodium	0.9946
35	L."Peel"	0.9714
34	L."Marlborough"	0.9288
32	L.grandiceps	0.8419
39	R.cinerea	0.8418
23	He.coralloides	1.0000
31	He.parvifolium	1.0000
28	He.intermedium	0.9490
24	He.depressum	0.9592
25	He.dimorphums	0.8410
36	Pseudognaphalium	0.8274
19	Ha.pulvinaris	0.9102
20	Ha.sinclairii	0.8240
14	G.involucratum	0.9623
18	G.umbricola	0.9000
5	A.triplinervis	0.8118
49	Genus"Z"	0.8058
29	He.lanceolatum	0.7913
1	A.keriensis	0.9668
4	A.trinervis	0.9582
3	A.subrigida	0.9474
2	A.rupestris	0.7386
27	He.filicaule	0.0000

Cophenetic correlation = 0.741

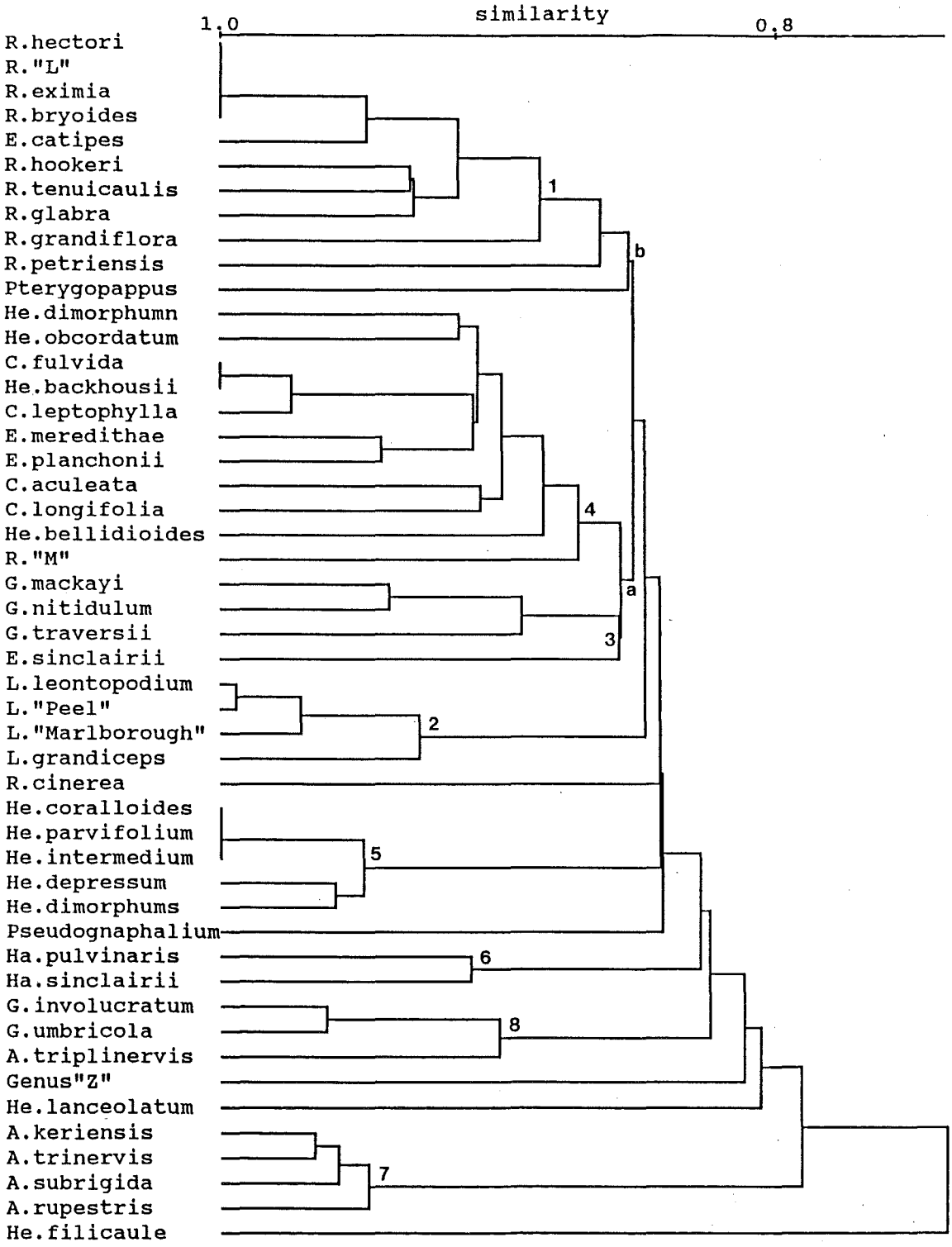


Figure 2.3. Single linkage phenogram from anatomical data with Gower's coefficient.

Helichrysum obcordatum and *H. dimorphum* (normal leaf) at 0.91. The Tasmanian species of *Cassinia* join them at 0.90. *Raoulia* "M" and *Helichrysum bellidioides* join separately.

Within cluster b, *R. grandiflora*, *Raoulia petriensis* and *Pterygopappus lawrencii* join the remaining species singly and at increasing levels of isolation (0.89, 0.86, and 0.85). There are two distinct clusters which join one another at 0.91. One is formed by the species of *Raoulia* subg. *Raoulia* (*R. glabra, R. hookeri and R. tenuicaulis*) and the other by *R. hectori, R.* "L", *R. eximia, R. bryoides* and *E. catipes*.

The cophenetic correlation coefficient has the value of 0.741. The similarity matrix is given in Appendix 6.

Comparison of the two phenograms

The clusters formed at higher similarity levels are almost the same. Differences occur mainly at low similarity levels. There are 8 almost consistent clusters. Cluster 1 includes *Ewartia catipes* and all species of *Raoulia* except *R. petriensis, R.* "M" and *R. cinerea*. Cluster 1 is in both phenograms associated with *Pterygopappus lawrencii* and *R. petriensis*. Cluster 2 comprises the species of *Leucogenes*. In cluster 3 *Gnaphalium mackayi, G. nitidulum* and *G. traversii* are united with each other and more remotely with *Ewartia sinclairii*. Cluster 4 is formed by *Cassinia*, the Tasmanian species of *Helichrysum* and *Ewartia* except *E. catipes, Helichrysum bellidioides, Helichrysum dimorphum* (normal leaf) and *Raoulia* "M". In the phenogram obtained by UPGMA, *Helichrysum bellidioides* is paired with *Raoulia* "M", while both of them have isolated positions within cluster 4 in single linkage clustering. In UPGMA, the paired *Ewartia meredithae* and *E. planchonii* are joined with the paired *Helichrysum obcordatum* and *H. dimorphum* (normal leaf) before they are united with the cluster of the New Zealand species of *Cassinia* and *Helichrysum backhousii*, while in single linkage clustering the paired species of *Ewartia* are joined with the New Zealand *Cassinia* species and *Helichrysum backhousii* before they are united with the cluster of *Helichrysum obcordatum* and *H. dimorphum* (normal leaf). Cluster 5, the inverse-dorsiventral species of *Helichrysum*, cluster 6, the species of *Haastia* and cluster 7, the New Zealand species of *Anaphalis*, are distinct clusters in both phenograms, with a minor

difference only within cluster 5. The last cluster, cluster 8, is formed by *Gnaphalium involucratum*, *G. umbricola* and *Anaphalis triplinervis*. The remaining species have isolated positions with changing affinities. *Helichrysum filicaule* is the most isolated species in both phenograms. *Helichrysum lanceolatum* joins *Haastia* in UPGMA clustering, but does not show close affinities with any species in single linkage clustering. Genus "Z" is isolated in both phenograms. *Pseudognaphalium luteoalbum* has no close affinities in single linkage clustering, but has distant links to cluster 4 in the phenogram obtained by UPGMA clustering. *Raoulia cinerea*, the last one of these isolated species, has no links with other species in the phenogram produced by single linkage clustering, but has distant affinities in UPGMA clustering with *Leucogenes*, *Ewartia catipes*, *Pterygopappus lawrencii* and the remainder of *Raoulia* except *R.* "M".

Considering now the genera, only *Leucogenes* and *Haastia* form distinct generic clusters. The New Zealand species of *Anaphalis* are quite distant from *Anaphalis triplinervis*. *Helichrysum* is represented in this study by 10 species in two sections. The species of *Helichrysum* sect. *Xerochlaena* have no close affinities with one another. The New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum* and *H. dimorphum* (normal leaf), cluster together. *H. lanceolatum* has no close links to other species. One of the Tasmanian species of this section clusters with the New Zealand *Cassinia* and the other one with *H. dimorphum* (normal leaf). The Tasmanian species of *Cassinia* form a cluster on their own. The Tasmanian species of *Ewartia* are clearly separated from the New Zealand *Ewartia*. But not even the Tasmanian species form a coherent association. *E. meredithae* and *E. planchonii* are clustered together while *E. catipes* has links to *Raoulia* subg. *Psychrophyton*. The species of *Gnaphalium* are split into two distinct groups. One group is formed by *G. involucratum* and *G. umbricola*, the other one by *G. mackayi*, *G. nitidulum* and *G. traversii*. All *Raoulia* species except *R. cinerea* and *R.* "M" have affinities with one another. They are divided into two isolated species, *R. grandiflora* and *R. petriensis* and two distinct groups, one of them comprising the species of *Raoulia* subg. *Raoulia*, the other one uniting the species of *Raoulia* subg. *Psychrophyton*.

2.4.4. Cladistic analyses

This analysis presents the first tentative approach to a cladistic study of the New Zealand and Tasmanian Gnaphaliinae.

The limit of 100 equally parsimonious trees was reached, the maximum permissible value of option MAXTREE.

The cladogram presented in Figure 2.4 is chosen from a careful evalution of the supporting characters in comparison with the conflicting characters supporting other equally parsimonious solutions.

The length of the cladogram is 96 steps with a consistency index of 0.375.

Black boxes on the branches represent synapomorphies that do not appear on other branches as reversals or parallelisms. Open boxes represent characters that reverse chainlike on branches higher up in the cladogram and the actual reversals are shown as crosses. Parallel lines indicate parallelisms, i.e., independent gains on different branches of the cladogram.

The characters used are shown in Appendix 3. The consistency index of each character is given. The characters with the highest consistency indices are the following: the shape of the epidermis cells, the presence or absence of large middle cells, whether the poorly differentiated mesophyll consists of oval/round/oval or oval/round cells, the exclusively abaxial position of the palisade cells and the position of sclerenchyma caps. The characters with the lowest consistency indices are the size of the substomatal chambers and the shape of the palisade cells.

The major clade is characterised only by the epidermis being equally thick on both sides, but this character reverses higher up in the cladogram. Also further up the tree the characters defining a clade are always reversed higher up in the cladogram.

The first well characterised clade is shown separately in Figure 2.5. The characters that identify this clade represent synapomorphies for this clade but symplesiomorphies within the Gnaphaliinae. These synapomorphies include the presence of palisade parenchyma in the midrib, absence of adaxial collenchyma, substomatal chambers other than medium and a lamina other than normal dorsiventral. Within this clade, *Raoulia glabra*, *R. hookeri* plus *R. tenuicaulis* form a monophyletic group, characterised by the presence of large middle cells. *Raoulia eximia*, *R. bryoides*, *R.* "L", *R. hectori* plus *Ewartia catipes* form also a monophyletic group, characterised

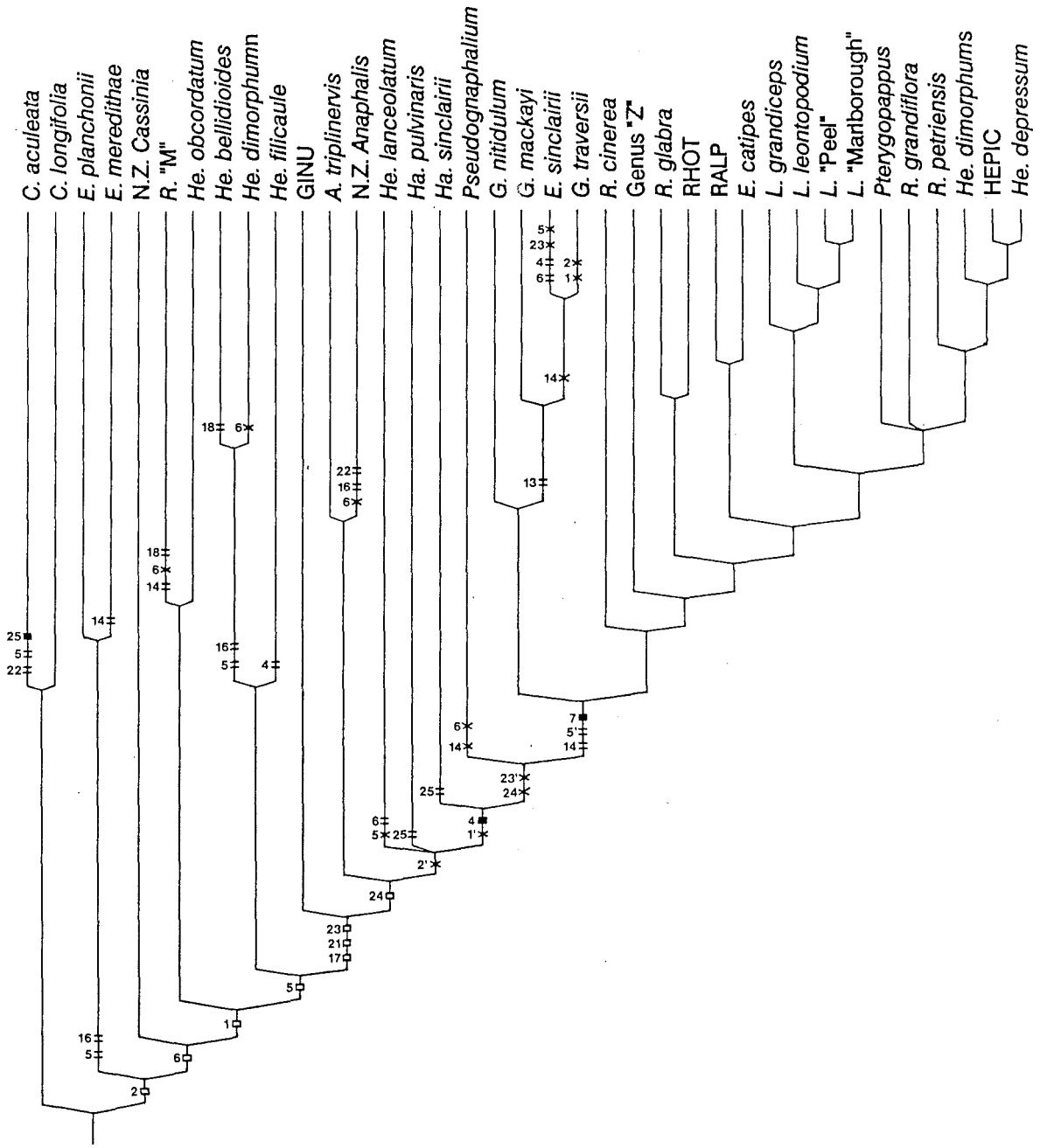


Figure 2.4. Working cladogram.

Black boxes = synapomorphies, open boxes = synapomorphies with reversals in the cladogram, crosses = reversals, and parallel lines = parallelisms.
GINU = *G. involucratum* & *G. umbricola*; RHOT = *R. hookeri* & *R. tenuicaulis*; RALP = *R. bryoides, R. eximia, R. hectori* & *R.* "L"; HEPIC = *H. coralloides, H. intermedium* & *H. parvifolium; He. dimorphumn* = *H. dimorphum* (normal leaf); *He.dimorphums* = *H. dimorphum* (scale-like leaf).

2	adaxial epidermis thicker than abaxial epidermis	2'	adaxial epidermis as thick as abaxial epidermis
6	substomatal chambers medium	1'	adaxial cuticle as thick as abaxial cuticle
1	adaxial cuticle thicker than abaxial cuticle	4	equal numbers of stomata on both sides
5	stomata slightly raised	24'	palisade parenchyma in lateral ribs present
17	adaxial collenchyma in midrib present	23'	collenchyma in lateral ribs absent
21	palisade parenchyma in midrib absent	5'	stomata level
23	collenchyma in lateral ribs present	14	palisade-like cells
24	palisade parenchyma in lateral ribs absent	7	normal spongy parenchyma absent

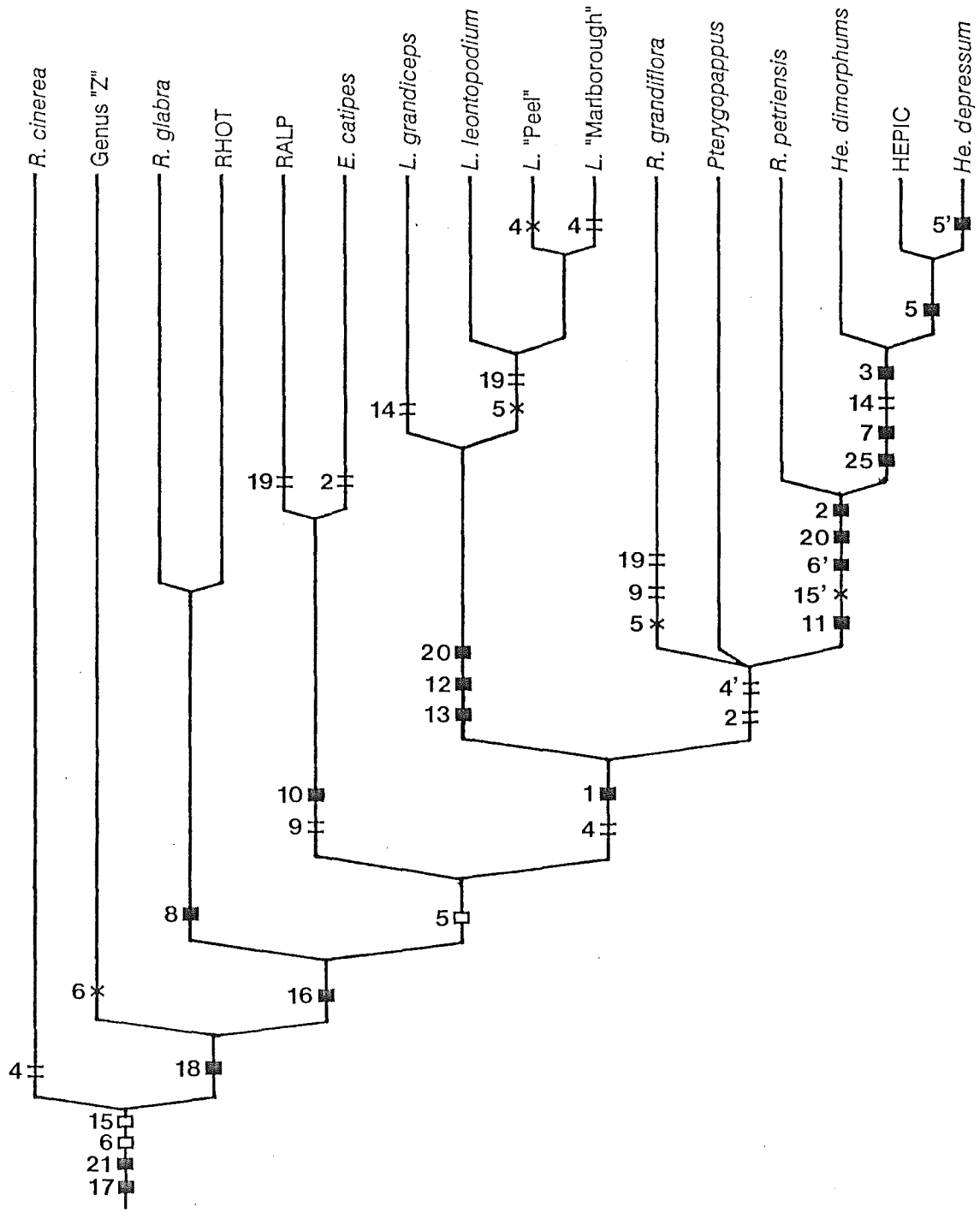


Figure 2.5. Detail of working cladogram.

Black boxes = synapomorphies, open boxes = synapomorphies with reversals in the cladogram, crosses = reversals, and parallel lines = parallelisms.
RHOT = *R. hookeri* & *R. tenuicaulis*; RALP = *R. bryoides, R. eximia, R. hectori* & *R.* "L"; HEPIC = *H. coralloides, H. intermedium* & *H. parvifolium; He. dimorphumn* = *He. dimorphum* (normal leaf); *He. dimorphums* = *H. dimorphum* (scale-like leaf).

17	adaxial collenchyma in midrib absent	11	palisade parenchyma present only on abaxial side
21	palisade parenchyma in midrib present	15'	lamina dorsiventral
6	substomatal chambers small	6'	substomatal chambers large
15	lamina equifacial	20	sclerenchyma caps on abaxial side
18	abaxial collenchyma in midrib absent	2	abaxial epidermis thicker than adaxial epidermis
16	midrib not protruding	25	scale-like leaves
5	stomata slightly raised	7	normal spongy parenchyma
4	stomata more numerous on adaxial side	14	palisade cells rod-shaped
1	abaxial cuticle thicker than adaxial cuticle	3	abaxial epidermis cells mostly rectangular
2	adaxial epidermis thicker than abaxial epidermis	5	stomata raised
4'	stomata present only on the adaxial side	5'	stomata extremely raised

by their poorly differentiated mesophyll. The robust clade of *Leucogenes* is defined by the presence of sclerenchyma caps on the adaxial and abaxial side of the midvein, by medium-sized middle cells and by small round cells on the abaxial side. The final clade comprising *Raoulia petriensis* and the New Zealand species of *Helichrysum* sect. *Ozothamnus* [except *H. lanceolatum* and *H. dimorphum* (normal leaf)] is supported by four synapomorphies. These species have the palisade parenchyma only on the abaxial side. They have large substomatal chambers and the epidermis is thicker on the abaxial side. If they have sclerenchyma caps, they are present on the abaxial side. *Raoulia petriensis* forms the sister group of the *Helichrysum* species. The latter are characterised by scale-like leaves, normal spongy parenchyma, normal palisade cells and rectangular abaxial epidermal cells.

The strict consensus tree is shown in Figure 2.6. The tree is characterised by two polychotomies at the bottom and relatively well-defined clades higher up the tree. The clade of Figure 2.5 is almost identical to the relevant part in the consensus tree. The only differences are that Genus "Z", *Raoulia cinerea* and the remaining species form a trichotomy and that the identical *Raoulia hookeri* and *R. tenuicaulis* do not form a monophyletic group with *R. glabra*, but also a trichotomy with the remaining species.

In considering the genera, only *Leucogenes* is a monophyletic genus. *Anaphalis* is in the working cladogram monophyletic, but in the consensus tree, the identical New Zealand species of *Anaphalis* and also the Himalayan *Anaphalis triplinervis* belong to the second polychotomy. In the working cladogram the New Zealand species of *Helichrysum* are split into three groups. One monophyletic group is formed by the species of *Helichrysum* sect. *Xerochlaena* (*H. bellidioides* and *H. filicaule*) plus *Helichrysum dimorphum* (normal leaf). In the consensus tree these are all members of the first polychotomy. The New Zealand species of *Helichrysum* sect. *Ozothamnus* [*H. coralloides, H. intermedium, H. parvifolium, H. depressum* and *H. dimorphum* (scale-like leaf)], except *H. lanceolatum*, form the second monophyletic group. *H. lanceolatum* stands on its own. The Tasmanian species of *Helichrysum* sect. *Ozothamnus* are members of the first polychotomy. In the working cladogram they do not form a monophyletic group either with any other *Helichrysum* species or with one another. In the working cladogram *Gnaphalium* forms two monophyletic groups, one of them comprising *G. nitidulum, G. mackayi* plus *G. traversii* and the second one only *G. involucratum*. In the

working cladogram all *Gnaphalium* species are included in the second polychotomy. In *Ewartia*, only *E. meredithae* plus *E. planchonii* is monophyletic in the working cladogram. They are both members of the first polychotomy. The two species of *Haastia* are not a monophyletic group. The genus *Raoulia* is split into several groups. The species of *Raoulia* subg. *Psychrophyton* except *R. grandiflora* (i.e., *R. eximia, R. bryoides, R.* "L" and *R. hectori*) are identical. *R. hookeri* plus *R. tenuicaulis* form a monophyletic group with *R. glabra* in the working cladogram. The remaining species of *Raoulia* (*R. petriensis, R. cinerea* and *R.* "M") have different affinities.

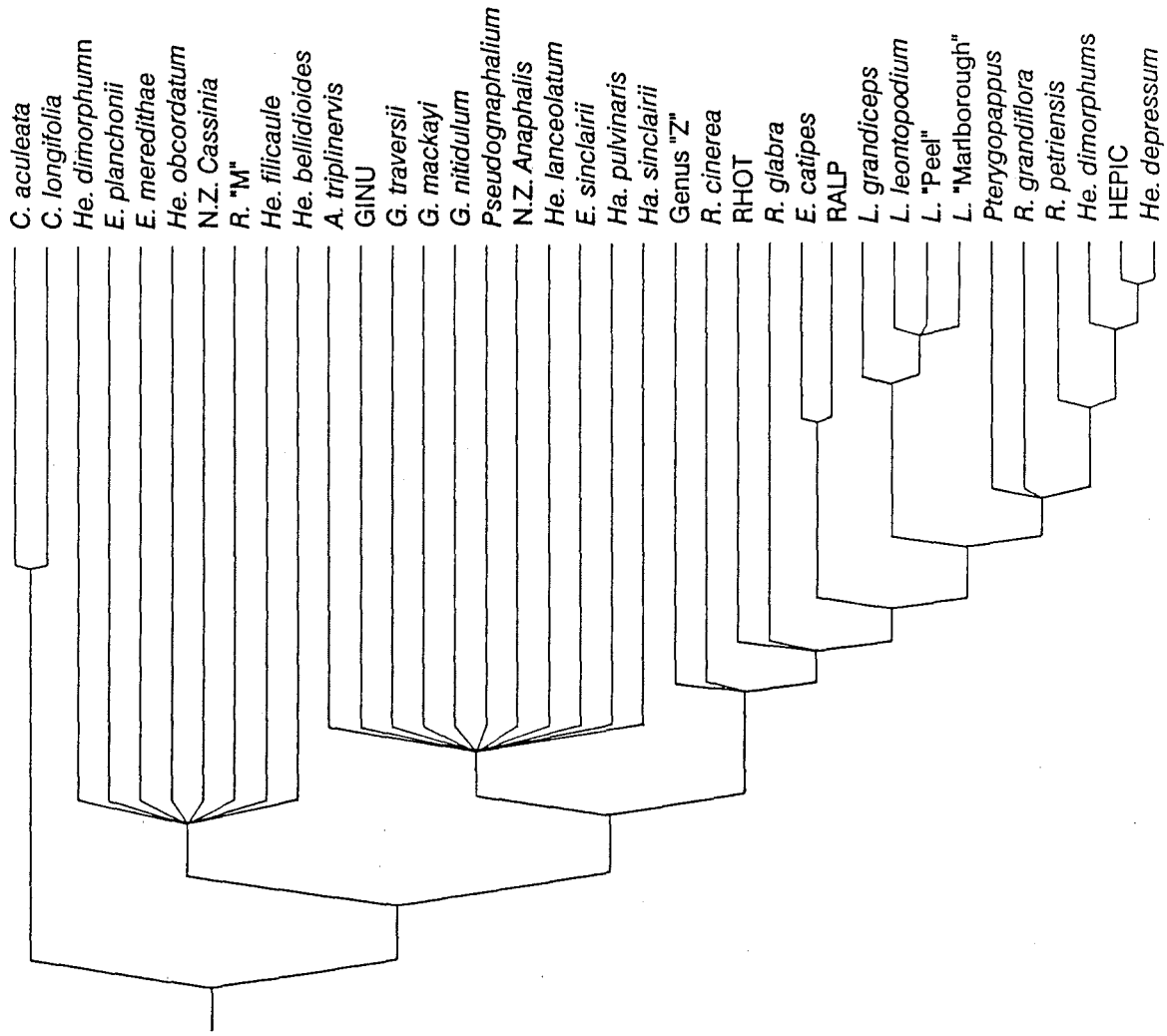


Figure 2.6. Strict consensus tree.
GINU = *G. involucratum & G. umbricola*; RHOT = *R. hookeri & R. tenuicaulis*; RALP = *R. bryoides, R. eximia, R. hectori &* R. "L"; HEPIC = *H. coralloides, H. intermedium & H. parvifolium; He. dimorphumn* = *H. dimorphum* (normal leaf), *He. dimorphums* = *H. dimorphum* (scale-like leaf).

2.5. Discussion

Environmental adaptation and leaf anatomy

The extreme habitats of some New Zealand Gnaphaliinae and some of their peculiar growth forms had by the turn of the century inspired botanists to undertake anatomical studies and to correlate anatomical features with the habitats of the plants. Lazniewski, for example, in 1896 briefly described the leaf anatomy of *Haastia pulvinaris*, *Ozothamnus microphyllus* and *O. selago* (= *Helichrysum parvifolium* and *Helichrysum intermedium*) to demonstrate the correlation with their xeric habitat. Characters explained as caused by the xeric habitat (e.g., Hauri, 1916; Cutler, 1978) have been the sclerenchyma caps of *Raoulia* subg. *Psychrophyton* and of the inverse-dorsiventral leaves of *Helichrysum*, and the water-storage cells of *Raoulia* subg. *Raoulia*. Other possible adaptations are compact mesophyll and equifacial mesophyll, which are, according to Pyykkö (1966), exclusively dependent on solar radiation.

According to Cutler (1978), there have in the past been too many studies, such as that of Haberlandt in 1896, where the authors ascribed adaptive properties to anatomical structures without any attempt at experimentation. Metcalfe (1983) argues that the ecological significance of characters has to be carefully interpreted by considering the heredity and taxonomy of the plants, together with any special features of their mineral nutrition. He points out that it is unusual, for example, to find all putative xeromorphic characters occurring together in one species. A dense hair covering correlates normally with raised rather than sunken stomata and with a thin rather a thick cuticle. This may be due to differences in the hereditary potential of xeromorphic plants with different taxonomic affinities.

In this survey of the Gnaphaliinae characters which may have evolved in relation to the environment are regarded as genetically controlled. Characters regarded as taxonomically useful in other comparative leaf anatomy studies (e.g., Pyykkö, 1966; Anderson, 1975; Keating, 1982, 1984) and in the work of Metcalfe and Chalk (1950), Napp-Zinn (1974) and Cutler (1978) were recorded and evaluated carefully. The characters used in the present study do not vary in a species between the two plants of different origin. Handsections of plants of ten species grown

in the glasshouses demonstrated the consistency within species. Therefore, differences in leaf anatomy in the Gnaphaliinae have a genetic basis and consequently potential taxonomic value.

Taxonomic implications of leaf anatomy characters

The only taxon with almost homogeneous leaves is *Pterygopappus lawrencii*, a monotypic Tasmanian genus which was examined because Merxmüller *et al.* (1977) tentatively included it in their *Gnaphalium* group, proposing relationships among *Gnaphalium* sect. *Euchiton*, *Leucogenes*, *Raoulia*, *Ewartia* and possibly the type species of *Haastia*. *Pterygopappus lawrencii* is characterised by its homogeneous leaf structure, adaxial stomata and a cuticle which is thicker on the abaxial than on the adaxial side. The epidermis is also thicker on the abaxial than on the adaxial side. The lamina structure most similar to that of *Pterygopappus lawrencii* is found in the species of *Raoulia* subg. *Psychrophyton* and in *Ewartia catipes*. In the numerical analyses *P. lawrencii* is most similar to *Ewartia catipes* (0.85), followed by *Raoulia petriensis* (0.84), *R. tenuicaulis* (0.83) and *R.* "L" (0.82). Hauri (1916) mentioned similar xeric adaptations in *P. lawrencii* and *R. bryoides* and also in *P. lawrencii* and *R. petriensis*. Thus the leaf anatomy supports Merxmüller *et al.*'s placement of *Pterygopappus*.

All species of *Raoulia*, except *R. cinerea* and *R.* "M", have equifacial or almost equifacial leaves. In both phenetic analyses they form a distinct cluster. Within this cluster the species of *Raoulia* subg. *Raoulia* form one group, characterised mainly by huge cells in the middle of the leaf with small oval or round cells on both sides. The huge thin-walled cells serve as water storage cells. As early as 1896, Diels explained them as an adaptive character enabling plants to survive the dry period of the frosts during July. Foweraker (1917) found these water storage cells in *R. tenuicaulis, R. australis* Hook. f. (= *R. hookeri* Allan), *R. lutescens, R. haastii, R. glabra* and *R. monroi*. Such cells seem therefore to be group specific for *Raoulia* subg. *Raoulia*.

The species of *Raoulia* subg. *Psychrophyton*, except *R. grandiflora*, form the second group. They have a similarity value of 1.0. Their leaf structure is almost homogeneous, with small oval cells on both the adaxial and the abaxial sides and small round cells in the middle. Sclerenchyma caps occur on either side of the veins and are said to be an adaptation to the

alpine environment (Hauri, 1916). Solbrig (1960), in his studies of cleared leaves, recorded sclerenchyma in all *Raoulia* species. The present study found them in the species of *Raoulia* subg. *Psychrophyton* only. Foweraker (op. cit.) did not mention sclerenchyma in the species of *Raoulia* subg. *Raoulia*. Solbrig described, for example, a sclerenchyma sheath being at least as thick as the bundle proper in *R. glabra*. Sclerenchyma sheaths around the bundle were observed in the present study in none of the species of *Raoulia* subg. *Raoulia* and have not been reported from anybody else studying the leaf anatomy of species of the Gnaphaliinae. Therefore, in spite of Solbrig's observations, the presence or absence of sclerenchyma caps is regarded as a useful character for distinguishing the species of *Raoulia* subg. *Psychrophyton* from the species of *Raoulia* subg. *Raoulia*.

In the cluster analyses, *R. grandiflora* and *R. petriensis* join the two *Raoulia* groups separately. The lamina structure of *R. grandiflora* is quite similar to that of the other species of *Raoulia* subg. *Psychrophyton* (*R. bryoides, R. eximia, R. hectori* and *R.* "L"), but instead of having a mesophyll consisting of small oval-round-oval cells, *R. grandiflora* has oval cells on the adaxial side and round cells on the abaxial side. *R. grandiflora* also has sclerenchyma caps. Therefore *R. grandiflora* seems to be anatomically more related to the species of *Raoulia* subg. *Psychrophyton* than to those of *Raoulia* subg. *Raoulia*. *R. petriensis*, which in the numerical analysis joins the *Raoulia* cluster but is most similar to *Pterygopappus lawrencii*, belongs to a different group of lamina structure types. It has inverse-dorsiventral leaves with stomata on the adaxial side and palisade-like cells on the abaxial side. Sclerenchyma caps were not observed.

The leaf anatomy of the *Raoulia* species supports therefore the division of the species currently included in *Raoulia* into two main groups. Ward (1981) pointed out that the pulvinate species of subgenus *Psychrophyton* form a coherent, uniform group, quite separate from *Raoulia* subg. *Raoulia*, which itself forms a coherent if internally variable entity, provided that *R. cinerea* and perhaps *R.* "M" are removed. In the present study, the leaf anatomy of *R. hectori* is very similar to the leaf anatomy of the pulvinate species of *Raoulia* subg. *Psychrophyton*. *R.* "M" and *R. cinerea* are quite dissimilar to both groups. Ward (op. cit.) suggests the exclusion of *R.* "M" from *Raoulia*. *R.* "M" has in the present study its highest similarity with *Ewartia planchonii*. It is the only species of those currently included in *Raoulia* which has normal dorsiventral leaves. *R. cinerea* has no close affinities to any of the other

species, thus supporting Ward's suggestion (op. cit.) to exclude it from *Raoulia*. *R.* "M" and *R. cinerea* are the only species of *Raoulia* with a protruding midrib, which underlines their dissimilarity from the other *Raoulia* species.

The only taxon, other than *Pterygopappus lawrencii*, which is included in the cluster of the *Raoulia* species is *Ewartia catipes*. *E. catipes* is in this study closely associated with the species of *Raoulia* subg. *Psychrophyton*. Their lamina structure is quite similar, but no sclerenchyma caps were observed in *E. catipes*. In the genus *Ewartia*, the species examined in the present study do not form a coherent group at all. The three Tasmanian species clearly belong to two separate groups. While *Ewartia catipes* is most similar to the species of *Raoulia* subg. *Psychrophyton*, the paired *E. meredithae* and *E. planchonii* have links to the Tasmanian *Helichrysum* sect. *Ozothamnus*, the New Zealand *Cassinia* and *Helichrysum dimorphum* (normal leaf). *E. meredithae* and *E. planchonii* both have normal dorsiventral leaves with stomata on the abaxial side. They are distinguished from each other only in the shape of the palisade cells. The sole New Zealand *Ewartia* species, *E. sinclairii*, has close affinities neither to the Tasmanian species of this genus nor to any other taxon of the Gnaphaliinae. Its lamina structure is quite similar to that of *Gnaphalium mackayi* and *G. traversii*. The lamina type is intermediate between equifacial and dorsiventral. Ward's numerical analyses in 1981 led to the hypothesis that *Ewartia sinclairii* is not congeneric with the Australian species of *Ewartia*. The leaf anatomy supports this view. Ward suggested in the same study that *E. catipes* and *E. planchonii* are closer to one another than to *E. meredithae*. The leaf anatomy does not support this; *E. planchonii* and *E. meredithae* are very similar and *E. catipes* is quite different.

In terms of lamina structure, Genus "Z" must be grouped with the *Raoulia* species, since it has equifacial leaves with stomata on both sides. In the numerical analyses, however, it is very isolated with no close affinities to any of the other taxa. This is not surprising since this taxon is the only one in the Gnaphaliinae with equal palisade parenchyma on both sides. Its midrib is protruding and some cells in the mesophyll are thick-walled. These characters do not show any similarity with either *Leucogenes grandiflora* or *Haastia sinclairii*, the two species to which it has been likened (Allan, 1961). Neither of these species has palisade parenchyma on both sides or

equal numbers of stomata on both sides as found in Genus "Z". Hybrid origin with *L. grandiceps* and *H. sinclairii* as parents, suggested also by Allan (op. cit.), is not supported by the leaf anatomy characters.

All species of *Leucogenes* have equifacial leaves with palisade parenchyma on the adaxial side, medium-sized middle cells, small round cells on the abaxial side and stomata either entirely or mostly on the adaxial surface or in equal numbers on both surfaces. These species also form a distinct cluster in the numerical analyses. They are not closely joined with other clusters, but there are distant links to the *Raoulia* cluster. *L. leontopodium*, *L.* "Marlborough" and *L.* "Peel" are the only species in this study which have sclerenchyma caps on both sides of the veins. *L. grandiceps* has no sclerenchyma caps at all. *L. grandiceps* is therefore clearly separated from the other three species, which are not identical but very similar to one another. In the phenograms of Ward (1981), the species of *Leucogenes* also form their own cluster.

The species of *Gnaphalium*, except *G. involucratum* and *G. umbricola*, have, together with *Ewartia sinclairii*, a type of leaf intermediate between equifacial and dorsiventral. They have stomata on the abaxial side, but the mesophyll is not divided into palisade and spongy parenchyma. All of them have storage cells of small size. The paired *Gnaphalium nitidulum* and *G. mackayi* are joined with *G. traversii* in the cluster analyses. *Gnaphalium involucratum* and *G. umbricola*, in contrast, have normal dorsiventral leaves with palisade parenchyma on the adaxial side and spongy parenchyma on the abaxial side. The midribs of these two species are greatly protruding while the midribs of the others protrude only slightly. With the separation of the anaphalioid species from *Gnaphalium* (Webb, 1987) and the exclusion of *Gnaphalium luteo-album* from *Gnaphalium* (Hilliard and Burtt, 1981), the indigenous *Gnaphalium* species in New Zealand all fall into sect. *Euchiton*. But the leaf anatomy suggests that not even the species of *Gnaphalium* sect. *Euchiton* form a homogeneous group.

All the remaining species in this study have either dorsiventral leaves with palisade parenchyma on the adaxial side and spongy parenchyma on the abaxial side or inverse-dorsiventral leaves with palisade parenchyma on the abaxial side and spongy parenchyma on the adaxial side.

All species of *Anaphalis* have normal dorsiventral leaves with the stomata on the abaxial side, but the New Zealand species of *Anaphalis* are clearly different from the Himalayan *Anaphalis triplinervis*. For example, the New Zealand species of *Anaphalis* have non-protruding ribs, while the ribs of *Anaphalis triplinervis* protrude greatly. The differences are also expressed in the phenograms, in which the New Zealand species of *Anaphalis* form a distinct cluster, while *Anaphalis triplinervis* is united with *Gnaphalium involucratum* and *G. umbricola*. The leaf anatomy supports the separation of the New Zealand species of *Anaphalis* from *Gnaphalium* (Webb, 1987), but they are not more similar to *Anaphalis* than to *Gnaphalium*. Therefore it has to be doubted whether this transfer was a significant improvement for the taxonomy of this group. According to the leaf anatomy, the New Zealand *Anaphalis* species seem to sit comfortably with neither *Anaphalis* nor *Gnaphalium*.

The species of *Helichrysum* sect. *Xerochlaena*, *H. bellidioides* and *H. filicaule*, also have normal dorsiventral leaves, but *H. filicaule* has some stomata on the adaxial as well as the abaxial side. *H. bellidioides* is most similar to *R.* "M", to the normal leaf of *H. dimorphum* and also to *H. obcordatum*, while *H. filicaule* has no close affinities, and is in fact the most isolated species within the Gnaphaliinae. Drury (1971) suggested that *H. bellidioides* is probably an anaphalioid cudweed, but it was not transferred by Webb (1987) into *Anaphalis* because it hybridises freely with other species currently treated in New Zealand within *Helichrysum*. The leaf anatomy underlines the relationship of *H. bellidioides* to some species of *Helichrysum*, but not to the species of *Anaphalis*. The possible relationship of *H. bellidioides* to *R.* "M" has not been recognised before and has therefore to be judged carefully. *H. filicaule* is without close affinities to any other taxa, including the other member of *Helichrysum* sect. *Xerochlaena*, *H. bellidioides*.

All *Cassinia* species have normal dorsiventral leaves with palisade cells and stomata on the abaxial surface, but the New Zealand species do not cluster with those of Tasmania. The New Zealand species of *Cassinia* cluster with the Tasmanian *Helichrysum backhousii* and are more similar to the Tasmanian *Ewartia planchonii*, *E. meredithae*, *Helichrysum obcordatum* and the New Zealand *H. dimorphum* (normal leaf) than they are to the Tasmanian *Cassinia* species. Hooker (1864) noted that *Cassinia fulvida* might be more correctly placed in *Ozothamnus* (= *Helichrysum*), and that *C. vauvilliersii* was scarcely distinguishable from a true *Ozothamnus* of Tasmania. The leaf anatomy definitely supports this opinion.

H. dimorphum (normal leaf) is the only New Zealand member of *Helichrysum* sect. *Ozothamnus* with close affinities to the Tasmanian species of this section. *H. lanceolatum* has an isolated position in the phenograms, which is underlined by its normal dorsiventral lamina with big cells in between palisade and spongy parenchyma and its bundle-sheath extensions. The remaining species of *Helichrysum* sect. *Ozothamnus*, characterised mainly by their inverse-dorsiventral lamina and adaxial stomata, form an isolated cluster without affinities to the Tasmanian species of this section, thus supporting Ward's proposal (pers. comm.) to separate the New Zealand group from *Helichrysum*. The *Helichrysum* species having inverse-dorsiventral leaves form two distinct groups. One group is formed by *H. coralloides*, *H. intermedium* and *H. parvifolium*. They are distinguished from the second group of *H. depressum* and *H. dimorphum* (scale-like leaf) by abaxial sclerenchyma caps. *H. depressum* has thick-walled cells on the adaxial side of the midvein, but no sclerenchyma caps. *H. dimorphum* (scale-like leaf) has also no sclerenchyma and is most similar to *H. depressum*, while the normal leaf is closest to the Tasmanian *H. obcordatum*. Apart from the different orientation, the mesophyll of both leaf types of *H. dimorphum* is very similar, but there are differences in the midrib, which is protruding in the normal leaf but not in the scale-like leaf. Wall (1920) suggested that this strange species with two leaf morphs might be a hybrid between *Helichrysum filicaule* and *H. depressum*. The anatomy of the scale-like leaf is quite similar to *H. depressum*, but the normal leaf is not similar to *H. filicaule*. The leaf anatomy studies cannot solve the problem of the origin of *H. dimorphum*, but indicate that if it is a hybrid, *H. filicaule* is an unlikely parent.

The two species of *Haastia*, *H. pulvinaris* and *H. sinclairii*, have normal dorsiventral leaves. They are paired in the phenograms and quite isolated from other species. Both have the stomata on the abaxial side and palisades on the adaxial side. The character which distinguishes *H. pulvinaris* from all the other species of the Gnaphaliinae is the presence of secretory ducts, probably resin ducts, abaxially to the veins. Secretory structures are expected to be particularly valuable as indicators of taxonomic affinity (e.g., Metcalfe and Chalk, 1950). The list of Napp-Zinn (1973) of Compositae species having secretory ducts does not mention any species of the Gnaphaliinae, nor does Drury and Watson's comparative anatomical study of the Inuloideae (1966). Therefore Merxmüller *et al.*'s proposal to include *H. pulvinaris* into the Gnaphaliinae finds no support in the leaf anatomy. Metcalfe states in 1983: "When canals or cavities occur in one species of a genus it is usually found that they occur in other species as well." Merxmüller *et al.* also suggest that *H. pulvinaris* is not congeneric with the other species of *Haastia*. Since the resin ducts are found in *H. pulvinaris* only and not in *H. sinclairii*, Merxmüller *et al.*'s proposal is strengthened by the leaf anatomy. Since the leaf tip of *Haastia pulvinaris* is quite peculiar, it is mentioned here. The adaxial side has a great number of projections of tissue, while the lower side has corresponding depressions. The palisade cells get smaller and more rounded towards the tip. Stomata are found on both surfaces. [For a detailed description see Lazniewski (1896) and Low (1899).]

Pseudognaphalium luteoalbum has dorsiventral leaves with palisade parenchyma on the adaxial side and spongy parenchyma on the abaxial side, but it has stomata on both sides. The irregular shape of the epidermal cells of both sides is distinctive. *Pseudognaphalium luteoalbum* has no close affinities to any of the other species of the Gnaphaliinae. Its highest similarity coefficient is with *Cassinia fulvida* and *Helichrysum backhousii* (0.84), but its similarity with the species of *Gnaphalium* is low. The leaf anatomy thus supports the separation of *Pseudognaphalium luteoalbum* from *Gnaphalium* (Hilliard and Burtt, 1981).

Cladistics

Much more information about the Gnaphaliinae, and especially about the New Zealand Gnaphaliinae and their Australian relations, would be necessary before establishing a well-founded evolutionary hypothesis. Therefore the cladistic analysis presented here has to be regarded as only the first exploratory attempt. Nevertheless it indicates some quite robust clades, and emphasises the value of anatomical characters.

The analysis was based on leaf anatomy characters only. Because quantitative (measurement) and ratio characters were excluded, the number of characters was not sufficient to achieve a fully resolved tree. Polychotomies had therefore to be accepted.

The consistency of character states may shed light on the value of a character. Characters with a high consistency index, e.g., the position of the sclerenchyma, are more "reliable" characters and may give a better indication of phylogenetic relationships than characters with a low consistency index. Those may be, for example, convergent characters but they may also be characters in which the states which have been delimited do not represent an evolutionary state. It is quite possible that the size of the substomatal chambers, with small medium and large, is not a good indication of ancestor-descendant relationship, though being constant within one species.

The analysis had to be based on the assumption that the New Zealand Gnaphaliinae and its Tasmanian related species are monophyletic. Bremer (1987) showed in his cladistic analysis that the Inuleae *sensu lato* are a paraphyletic group. He treated the Gnaphaliinae as a separate tribe from the Inuleae, called the Gnaphalieae. According to Merxmüller *et al.*(1977), the species of the Australian region of the Gnaphalium - Anaphalis - Helichrysum complex are closely connected. Therefore the assumption that the Gnaphaliinae are monophyletic seems to be justified.

The most useful operational method for assessing character state polarities is out-group comparison (Hennig, 1966; Platnick, 1979; Watrous and Wheeler, 1981; Maddison *et al.*, 1984; Mabee, 1989). The Tasmanian species of *Cassinia* were chosen as the out-group. It was shown in the phenetic analysis that the New Zealand species of *Cassinia* are not grouped with the Tasmanian species of this genus. Rather, the New Zealand species of *Cassinia* are related to the Tasmanian species of *Helichrysum* sect. *Ozothamnus*. Since the present study is concerned with

Merxmüller *et al.*'s (1977) *Gnaphalium*, *Anaphalis* and *Helichrysum* groups, the Tasmanian species of *Cassinia* were selected as the out-group. Merxmüller *et al.* did not include the genus *Cassinia* in their *Helichrysum* group. *Cassinia* (together with a few other genera) is mentioned separately. However, Merxmüller *et al.* stated that *Cassinia* could, at least technically, be included in *Helichrysum*. In the phenograms presented here, the Tasmanian species of *Cassinia* always form a cluster on their own. Therefore *Cassinia* seems to be the best option for the out-group.

Brief mention may be made of the fact that ANCESTOR rooting was also employed. The characters were polarised by assuming that the hypothetical ancestor had a normal dorsiventral leaf. The results are not presented or discussed here, except to mention that they were almost identical to the ones obtained by the method using out-group comparison.

More than 100 equally parsimonious trees were obtained. One reason is the lack of characters. A second reason is that the program constructs all possible most parsimonious trees for zero-length branches. Character weighting by using Farris's (1969) weighting function did not provide fewer equally parsimonious trees. Character weighting is not further discussed here. It was tried, but it did not improve the trees. Therefore it was decided to present the consensus tree of the first 100 equally parsimonious trees and also a working cladogram. It has to be noted again, that this cladistic analysis has to be regarded as the first exploratory attempt of analysing data of the New Zealand Gnaphaliinae cladistically and as a means of understanding trends in the evolution of the characters and the value of those characters.

Monophyletic groupings should be carefully studied in terms of shared derived states that link them together (Simpson, 1986). If these synapomorphies are questionable (e.g., defined by a single character state change showing reversal or convergence), then the monophyly of the group is questionable. Since the in-group of the present study is characterised only by an equally thick epidermis and this character state is reversed higher up in the tree, the monophyly of the study group has to be doubted.

All EUs included in the two polychotomies of the consensus tree have dorsiventral leaves. For a better resolution additional characters to the anatomical ones would be necessary. Because of the lack of characters it cannot even be stated that the group under study is not monophyletic. This possibility has to be kept in mind for any future cladistic analysis.

A well-defined clade is formed by *Raoulia cinerea* and the remaining species higher up the tree. This clade is relatively well-resolved and consistent. In considering this clade, this first cladistic attempt is quite informative. This clade is characterised by four synapomorphies, which are symplesiomorphies within the whole study group but are most parsimoniously interpreted as synapomorphies at this level. The species included in this clade are all species which are not dorsiventral or transitional dorsiventral.

In considering the genera, it is very interesting that the New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum*, are monophyletic and quite separated from the species of *Helichrysum* sect. *Xerochlaena* and the Tasmanian species of *Helichrysum* sect. *Ozothamnus*. This supports Ward's planned separation of the New Zealand species of *Helichrysum* sect. *Ozothamnus* from *Helichrysum*. Ward (1981) suggested that *Raoulia* might not be a monophyletic group. This is underlined by the cladistic analysis. The species of *Raoulia* subg. *Raoulia*, except *R.* "M", and also the species of *Raoulia* subg. *Psychrophyton*, except *R. grandiflora*, seem to be two monophyletic associations. *Raoulia* "M" seems to be not related to any of the *Raoulia* groups at all. *Raoulia petriensis* is the sister group to the New Zealand species of *Helichrysum* sect. *Ozothamnus*. It has its position between *Helichrysum* and *Raoulia*. *Ewartia* is not a monophyletic group. Therefore not only is the sole New Zealand species of *Ewartia* (*E. sinclairii*) probably not congeneric with *Ewartia*, but it has to be doubted that the Tasmanian species of *Ewartia* are congeneric. The genera included in the polychotomies of the consensus tree will not be discussed here since the information obtained is too meagre.

According to the cladogram (Figure 2.5), the New Zealand species of *Helichrysum* sect. *Ozothamnus* having inverse-dorsiventral leaves have the most derived character states of the Gnaphaliinae. It has to be noted that the normal leaf of *Helichrysum dimorphum* has ancestral while the scale-like leaf has derived character states. *Raoulia petriensis* has the most derived character states of the *Raoulia* species. The character states of *Raoulia grandiflora* are more derived than those of *Leucogenes*. The most ancestral character states of this clade has *Raoulia cinerea* followed by Genus "Z". These hypothesised relationships should be tested in cladistical analyses using more and independent characters.

The cladistic analysis provided useful information in several aspects. It demonstrated which characters are phylogenetically more or less "reliable". The lamina structure seems to be phylogenetically very important. The analysis suggests which of the currently accepted genera might be well defined (i.e., monophyletic) and which ones not and also provides a hypothesis concerning the phylogeny of some of the species.

The presented hypothesis is very weak and should not be taken as a precise phylogenetic hypothesis, but the results definitely suggest the value of a more detailed cladistic analysis with characters from other fields than anatomy.

2.6. Conclusion

A number of conclusions may be drawn from this study of the leaf anatomy of Gnaphaliinae.

The New Zealand species of *Anaphalis* form a separate group, quite different from the Himalayan *Anaphalis triplinervis*. The Tasmanian species of *Cassinia* are different from the New Zealand species of this genus. The New Zealand *Cassinia* is quite similar to the Tasmanian species of *Helichrysum* sect. *Ozothamnus*. The Tasmanian species of *Ewartia* are clearly different from the sole New Zealand species, and even the Tasmanian species of this genus are split into two entities. *Gnaphalium* forms two distantly related groups. The *Haastia* species are more similar to one another than to any other species. *Helichrysum* is split into the group with inverse-dorsiventral leaves and several species with different affinities. The species of *Leucogenes* are closely related. *Pseudognaphalium* is quite isolated. *Pterygopappus* is closest to *Raoulia*. *Raoulia*, except *R.* "M" and *R. cinerea,* forms one distinct association, which is divided into two groups. Genus "Z" stands isolated.

Leaf anatomical studies in part support present classification, in part Ward's (1981) hypotheses and in part new relationships. If the leaf anatomy supports either existing view, this is then more likely to be correct. If the leaf anatomy suggests new relationships, the results have to be judged carefully and require further research.

The cladistic analysis provided the first attempts at a phylogenetic hypothesis and may serve as a starting point for future phylogenetic investigations.

The results of this leaf anatomy survey of the Gnaphaliinae could be used to clarify intrageneric and intergeneric relationships in a revision of the current taxonomy of the group.

CHAPTER THREE

CHEMOTAXONOMY

3.1. Introduction

Considering that chemical data have been applied to taxonomic problems for only the past 30 years, an enormous amount of work has been published in chemotaxonomy and the value of this field is now recognised widely. The most commonly used of all secondary constituents in taxonomic studies are the flavonoids.

The flavonoids are one of the largest groups of naturally occurring phenols. Most phenolic nuclei are derived biosynthetically from 5-dehydroquinate via the shikimic acid pathway or from acetate via polyketide metabolism. Flavonoid variants are derived further from the pathways of flavonoid biosynthesis (Hahlbrock and Grisebach, 1975). The flavonoid aglycones, that is, the flavonoids without attached sugars, occur in plants in a variety of structural forms. All contain two aromatic rings linked by a three carbon unit which may or may not form a third heterocyclic ring (Markham, 1982).

Flavonoids are probably the most useful class of compounds for a taxonomic study, because they are chemically stable, widely distributed and have a strong tendency for taxonomically related plants to produce similar types of flavonoids (eg., Harborne, 1984; Harborne and Turner, 1984; Markham, 1982). Following Alston and Turner's first review on biochemical systematics in 1963, a number of reviews on the chemistry and systematics of flavonoids have produced extensive data on the chemistry, taxonomy and evolution of the flavonoids (eg. Harborne *et al.*, 1975; Giannasi, 1978; Crawford, 1978; Bohm, 1987). Early applications of flavonoid data in taxonomic research are found in the work of Bate-Smith (1958). Flavonoid data have now been included in so many taxonomic treatments throughout the whole plant kingdom, that only a selection can be mentioned here (Bohm *et al.*, 1986; Averett *et al.*, 1981-1986; Crawford *et al.*, 1986; Markham *et al.*, 1989).

A vast phytochemical literature exists on the biggest family of the angiosperms, the Compositae. Chemotaxonomic studies have been undertaken in various tribes at different levels of systematic enquiry (e.g., King, 1986; Crawford, 1981; Bain, 1985), but in spite of the increasing chemotaxonomic interest, the flavonoid chemistry of the Gnaphaliinae has not been extensively explored. Harborne (1977) reports in his chemical review on the Inuleae that most of the few flavonoid isolations have been undertaken on *Gnaphalium* and *Helichrysum* (e.g., Hänsel *et al.*, 1962, 1967, 1969, 1972; Candy *et al.*, 1975; Geissman *et al.*, 1967), but the information was not sufficient for taxonomic use. The most characteristic feature of Inuleae flavonoids was found to be the presence of flavonols lacking B-ring hydroxylation and Harborne concluded that *Gnaphalium* and *Helichrysum* have a very similar and complex pattern of flavonoids in their tissues. Little taxonomically oriented work has been published on the flavonoids of the Gnaphaliinae with only some isolated taxonomic studies at the species level or of flower pigments (e.g., Di Modica *et al.*, 1963; Douglas *et al.*, 1977). Hegnauer's chemistry update (1989) of his flavonoid data of Compositae (1964) reports isolations of many flavonoid compounds but no taxonomic treatments of the Gnaphaliinae.

This chapter is therefore, as far as is known, the first chemotaxonomic analysis of leaf flavonoids of the Gnaphaliinae at the genus level. It presents a preliminary flavonoid study of the New Zealand Gnaphaliinae and its Tasmanian relatives.

The aim of the studies reported here was to examine the distribution of the phenolic constituents within the Gnaphaliinae to provide data for use in taxonomic revision. This was attempted by comparing flavonoid profiles of 47 taxa using two-dimensional paper chromatography. Compounds with similar positions in two plants were co-chromatographed to check whether they were identical. Identification of some of the major flavonoid compounds was attempted by UV absorption spectroscopy and by hydrolysis and co-chromatography with authentic samples, but a detailed flavonoid analysis was beyond the scope of this project. The flavonoid data were analysed by visual comparison and by cluster analyses. It has to be emphasised again that the aim of this study was not to obtain biochemical information but rather to contribute information for clarifying intrageneric and intergeneric relationships in the Gnaphaliinae.

3.2. Materials and methods

Materials

Fresh plant material of most species was collected on field trips in the South Island (New Zealand) in 1987, in Tasmania in 1988 and in the North Island (New Zealand) in 1989. *Leucogenes* "Marlborough" and *Leucogenes* "Peel" were obtained from the experimental gardens of Botany Division, D.S.I.R., at Lincoln. *Anaphalis triplinervis* was grown in the glasshouses of the University of Canterbury, Department of Plant and Microbial Sciences, Christchurch, N.Z..

All plant material was collected between January and the end of March to minimise seasonal variation. Only mature, healthy leaves were used for analyses. Specimens of all taxa are deposited at the Herbarium of the University of Canterbury (CANU). Collecting data are given in Appendix 4.

The leaf material was preserved by freeze-drying in an Edwards (U.K.) 30P2 centrifugal freeze-dryer, then stored in sealed plastic bags.

Methods

Paper-Chromatography of Flavonoids

Flavonoids of all specimens collected were extracted from freeze-dried leaves and analysed by two dimensional paper chromatography (2D-PC) following the techniques of Mabry *et al.* (1970) and Markham (1982). 0.5 g of dry leaves were powdered in a mortar and pestle and the powder was homogenised first for one minute in 20 ml MeOH (9:1) and second for one minute in 20 ml MeOH (1:1) using an Ultra-Turrax homogeniser (Janke & Kunkel F.R.G.). At each step, the mixture was left for 6 hours. The extracts were filtered through Whatman No. 54 filter paper and the two extracts combined and reduced at 30°C *in vacuo* to 20% of the original volume. The resultant aqueous extract was extracted several times with chloroform until chlorophyll was removed, then evaporated to dryness in a rotary vacuum evaporator. The

extract was redissolved in 2 ml of 80% aq.methanol and 200 μl aliquots (i.e., the extract of 50 mg dry weight of foliage respectively) applied to 46 cm x 57 cm sheets of Whatman 3MM chromatography paper for descending paper chromatography in TBA (*t*-BuOH / HOAc / H_2O 3:1:1) followed by 15% aq. acetic acid in the second dimension. Developed sheets were examined under ultraviolet (UV) light (366 nm) before and after fuming with ammonia. Some sheets were observed in UV and daylight after spraying with a 1% solution of Naturstoffreagenz-A, Roth (NA; i.e., diphenyl-boric acid-ethanolamine complex) in methanol. BAW (n-BuOH / HOAc / H_20 40:10:22) was used also for some extracts as development solvent for the first dimension. Paper chromatograms having spots with low mobility in both solvent systems were rerun in 50% HOAc. R_f values (the distance travelled by the compound from the origin divided by the distance travelled by the solvent front from the origin) were calculated for each spot. Rutin was used as a marker compound.

Additional chromatographic procedures

PC on small paper sheets

Crude extracts were chromatographed on 20 cm x 20 cm sheets of Whatman 1MM chromatography paper for ascending chromatography using BAW as solvent for the first dimension and 15% HOAc for the second dimension.

Thin-layer chromatography (TLC)

Some extracts were chromatographed on cellulose thin-layers (TLC). Schleicher and Schuell Avicel cellulose sheets were used as well as glassplates spread with Merck microcrystalline "Avicel" cellulose. The solvents used for the first dimension were BAW, TBA or Forestal (HOAc / H_2O / HCl 30:10:3), for the second dimension 5%, 15% or 50% HOAc.

High Performance Liquid Chromatography (HPLC) Analysis

Reverse phase high-performance liquid chromatography (HPLC) was attempted. The procedures used are described in Appendix 5.

Co-chromatography

To check whether spots with identical R_f values and colours under UV light were really the same, they were cut out, eluted in 50% aq. methanol, evaporated *in vacuo* and co-chromatographed (i.e., chromatographing of the two flavonoids side by side on the same chromatogram, both separately and mixed) by cellulose TLC using three different standard solvents.

Larger scale flavonoid isolation

Larger quantities of flavonoids for chemical structure studies were isolated by 1D-PC on Whatman 3MM paper using either TBA or HOAc. The bands were cut out and eluted with 50% MeOH by descending chromatography. The eluate was evaporated *in vacuo*, then filtered and applied to a C-18 silica column, followed by a Sephadex LH-20 column and eluted with methanol.

Flavonoid identification techniques

Ultraviolet-visible Absorption Spectroscopy

Major spots of multiple chromatograms were cut out and dissolved in 5 ml AR methanol. Purified or hydrolysed flavonoids were dissolved in AR methanol. The solution was then diluted until the absorption level of the major peak was 0.6 A.U. (= Absorbance units). UV spectra were recorded on a Pye Unicam SP1800 double beam spectrophotometer before and after addition of either sodium methoxide, aluminium chloride, aluminium chloride / hydrochloric acid, sodium

tetraborate or sodium tetraborate / boric acid. The position of the absorption maximum was noted and by adding test reagents to the sample solution any resultant shifts in the absorption peaks were observed. An interpretation of the spectra was tried following the guidelines of Mabry *et al.* (1970) and Markham (1982).

Hydrolysis

Standard hydrolysis procedures were followed (Markham, 1982) to attempt to cleave the sugar from the aglycone. Either purified flavonoids were evaporated *in vacuo* to dryness or major spots from multiple chromatograms were cut out, dissolved in methanol, filtered and evaporated to dryness. The residue was dissolved in 3 ml MeOH and 1 ml of this solution hydrolysed with 2 ml of 2N HCl for 60 min on a boiling water bath. If acid-labile (e.g., some flavonols) aglycones were expected, 1 ml of the solution was partially hydrolysed by refluxing with 1M trifluoroacetic acid (TFA) for 30 min on a boiling water bath. The hydrolysates were evaporated to dryness in a rotary evaporator and the residue was dissolved in a minimum of 50% MeOH and chromatographed beside the unhydrolysed extract on TLC-plates with 15% HOAc to determine whether hydrolysis had taken place. If hydrolysis had occurred, identification of the resulting aglycones was attempted by UV spectroscopy and by 2D-TLC developed in standard solvents by comparison with authentic markers.

Survey of the species

At least one sample of each taxon was analysed by 2D-PC. Two to four samples of 14 taxa (see Appendix 4) were analysed by 2D-PC to check for constancy or intraspecific variation. Multiple chromatograms of the same extract of a few species were run to check for constancy of the minor spots. The additional chromatography methods were tried initially to find the best method for routine analysis.

Helichrysum intermedium, Cassinia fulvida and *Raoulia glabra* were subjected to full chemical study including large scale flavonoid isolation and identification by using UV absorption spectroscopy, hydrolysis and co-chromatography of the aglycone with authentic standards.

Major flavonoids of other species (*Helichrysum lanceolatum, H. depressum, H. dimorphum, Cassinia aculeata* and *Haastia pulvinaris)* were identified partially by UV absorption spectroscopy and hydrolysis. Major spots of all species were checked by co-chromatography.

Numerical analyses

To form the basic data matrix, 47 OTUs of the Gnaphaliinae were scored in binary code (0,1) for the presence and absence of 38 flavonoids. Where a spot was both present and absent within one taxon or the identity of one spot could not be proved, the state was entered as "no comparison".

Similarities among the species were calculated by the simple matching coefficient and as well by Jaccard's coefficient. The similarity values were clustered by the unweighted pair group method of arithmetic averages (UPGMA) and by the single linkage technique. The degree of fit of the phenogram to the similarity matrix from which it was derived was measured using the cophenetic correlation coefficient of Sokal and Rohlf (1962). The methods are explained in chapter 2.

The program used for the numerical analyses was "Gower", written by Drs. C.M. Frampton, G.A. Findlay and J.M. Ward, Christchurch.

3.3. Results

3.3.1. Paper chromatography

Samples of phenolics prepared from crude extracts of leaves of several taxa were examined initially by reversed-phase high-performance liquid chromatography (HPLC) as suggested by several authors (e.g., Heubl and Vogt, 1985; Park 1987). Unfortunately this technique proved impractical due to the large number of UV-absorbing components present in the chromatograms and the difficulty in resolving and identifying these. Two-dimensional paper chromatography (2D-PC) with large sheets proved to be much more practical for qualitative examination of leaf phenolics as this provided good resolution as well as permitting easier identification of phenolics by their R_f values and fluorescence characteristics. However, 2D-PC in standard solvent systems (TBA / 15% aq. acetic acid) could not separate compounds with low mobility in both solvents. Good resolution was obtained by rerunning such chromatograms with 50% aq. acetic acid. 2D-PC on small paper sheets and thin-layer chromatography (2D-TLC) were also tried, but they lacked adequate resolution.

Examination of the individual 2D-PCs under UV light revealed the presence of 65 different UV-fluorescent or absorbing spots. These were, with the exception of the 14 bright yellow fluorescent spots, probably phenolic or flavonoid in nature (Markham, 1982). Figure 3.1 shows the master composite chromatogram derived from all the individual chromatograms. On the 2D-PCs all spots relevant to the present study typically appeared dark purple or yellow, and they are distinguished by their colour changes in NH_3 vapour and by their R_f values in TBA, BAW, 15% aq. acetic acid and 50% aq. acetic acid. Other blue fluorescent spots were encountered in almost all of the taxa. They are probably simpler phenolics such as cinnamic acids and their glycosides. Their 2D-PC pattern is often inconsistent and for this reason no attempt has been made in this study to use these components in a taxonomic interpretation. The only compound appearing as a red spot was an anthocyanin, identified as a cyanidin-O-glucoside. It was found in high concentrations in the leaves of *Raoulia* "M", *R. cinerea*, *R. tenuicaulis* and *R. hookeri* and in lower concentration in *R. glabra, R. petriensis, Helichrysum bellidioides, Gnaphalium mackayi* and *G. traversii*.

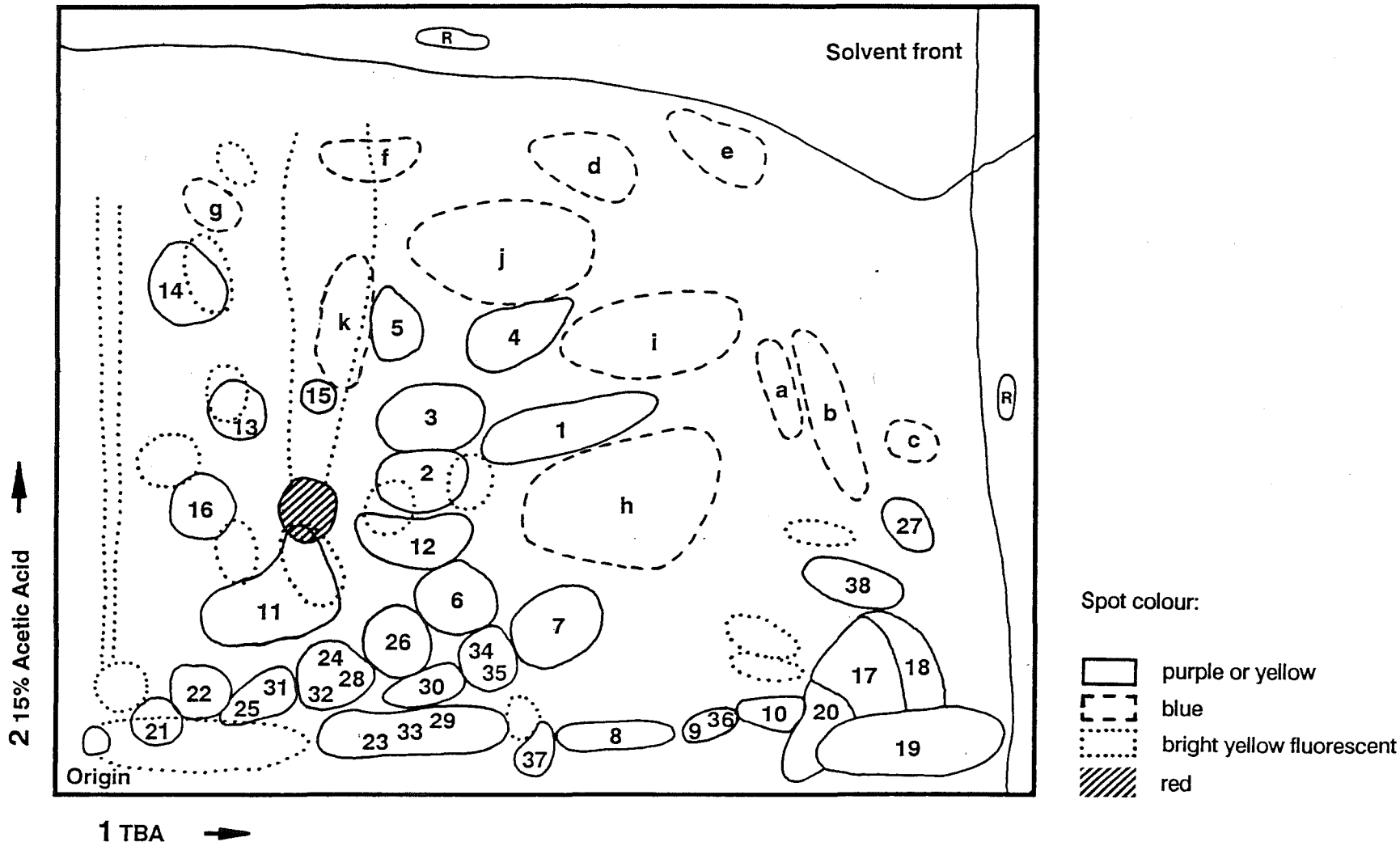


Figure 3.1. Master composite chromatogram of all components.

This anthocyanin was not included in the analysis since it could not be proved that it had taxonomic value. Seasonal induction of anthocyanin biosynthesis in some *Raoulia* spp. has been reported already by Foweraker (1916), but a thorough study in this field was beyond the scope of this project.

Fluorescence characteristics and R_f values of the flavonoids are given in Table 3.1. Figure 3.2 shows the master chromatogram of the flavonoids run in TBA / 15% HOAC, while Figure 3.3 shows the master chromatogram of the flavonoids run in TBA / 50% HOAc. Only the spots with low mobility can be seen, since all the other spots ran with the solvent front. Table 3.2 presents the semi-quantitative distribution of the different flavonoid compounds in the various species examined. Relative intensities of spots refer only to the pattern of spots for the species under consideration and are not comparable between the taxa. Thus, the most intense spot(s) in each species are rated "+ + +" irrespective of their intensity relative to spots in the PCs of other species.

As can be seen (Table 3.2), all of the 47 taxa examined are distinguished by their flavonoid patterns, although in some cases the differences are minor or of a quantitative nature only.

Intraspecific variation might limit the value of the flavonoids as species markers (eg., Bohm, 1987; Williams and Harvey, 1982). Since the purpose of this study was not to clarify specific but rather generic limits, intraspecific flavonoid variation was examined to only a limited extent; it was found to be either absent or minor and of a quantitative rather than a qualitative nature. Only the presence or absence of minor spots varied. The spots of *Haastia sinclairii, Helichrysum coralloides, H. depressum* and *Leucogenes leontopodium* entered in Table 3.2 as "?" were minor spots which were present in one specimen and absent in another. Traces of spots were also sometimes present or absent on multiple PCs obtained by running the same amount of the same extract. The spots were recorded in those cases as present.

Table 3.1. R_f values and fluorescence characteristics of flavonoids.

Spot#	Colour under UV	Colour under UV +NH_3	R_f value (x100)in TBA	R_f value (x100)in BAW	R_f value (x100)in 15%HOAc	R_f value (x100)in 50%HOAc
1	purple	green	47	55	45	
2	purple	green	37	48	39	
3	purple	green	38	48	48	
4	purple	green	45	52	59	
5	purple	green	33		61	
6	purple	green	40		20	
7	purple	green	52		16	
8	yellow	yellow	60		2	
9	purple	yellow	68		2	
10	purple	purple	77		2	
11	purple	yellow	20		20	
12	purple	yellow	37		30	
13	purple	green	16		47	
14	purple	green	12		65	
15	purple	purple	27		51	
16	purple	yellow	15		33	
17	purple	green	85	85	2	
18	purple	purple	90	90	10	
19	yellow	yellow	90	95	2	
20	yellow	yellow	80	90	2	
21	purple	green	23		>10	6
22	purple	green	37		>10	15
23	yellow	yellow	27		>10	42
24	yellow	yellow	55		>10	33
25	purple	yellow	41		>10	23
26	purple	purple	73		>10	37
27	purple	purple	92		42	
28	purple	yellow	51		>10	27
29	purple	green	55		>10	42
30	purple	green	61		>10	37
31	yellow	yellow	41		>10	23
32	yellow	yellow	59		>10	30
33	purple	yellow	63		>10	43
34	yellow	yellow	65		>10	52
35	purple	yellow	69		>10	50
36	purple	purple	68		2	
37	yellow	yellow	37		2	
38	purple	purple	84		25	

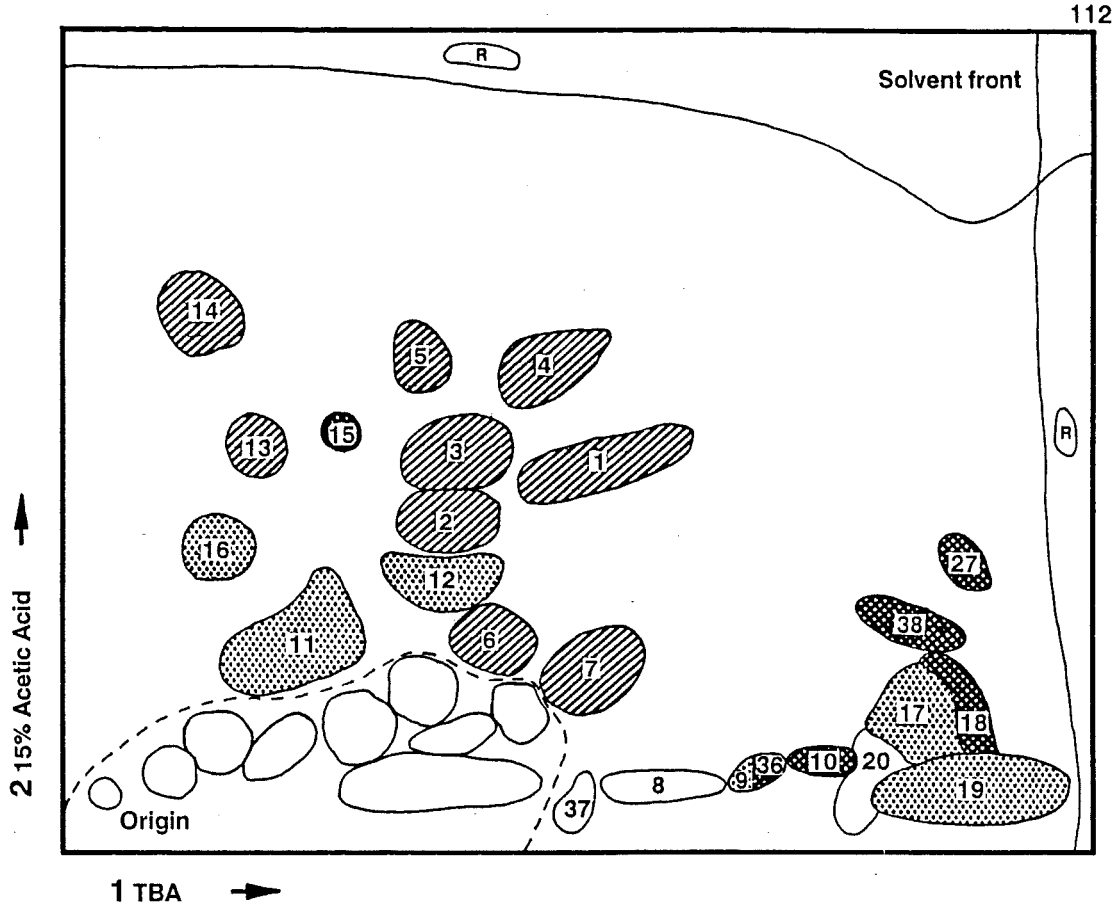


Figure 3.2. Master composite chromatogram of flavonoids (TBA / 15% HOAc).

Spot colour before and after spraying with NH_3:

purple / green
purple / yellow
purple / purple
yellow / yellow

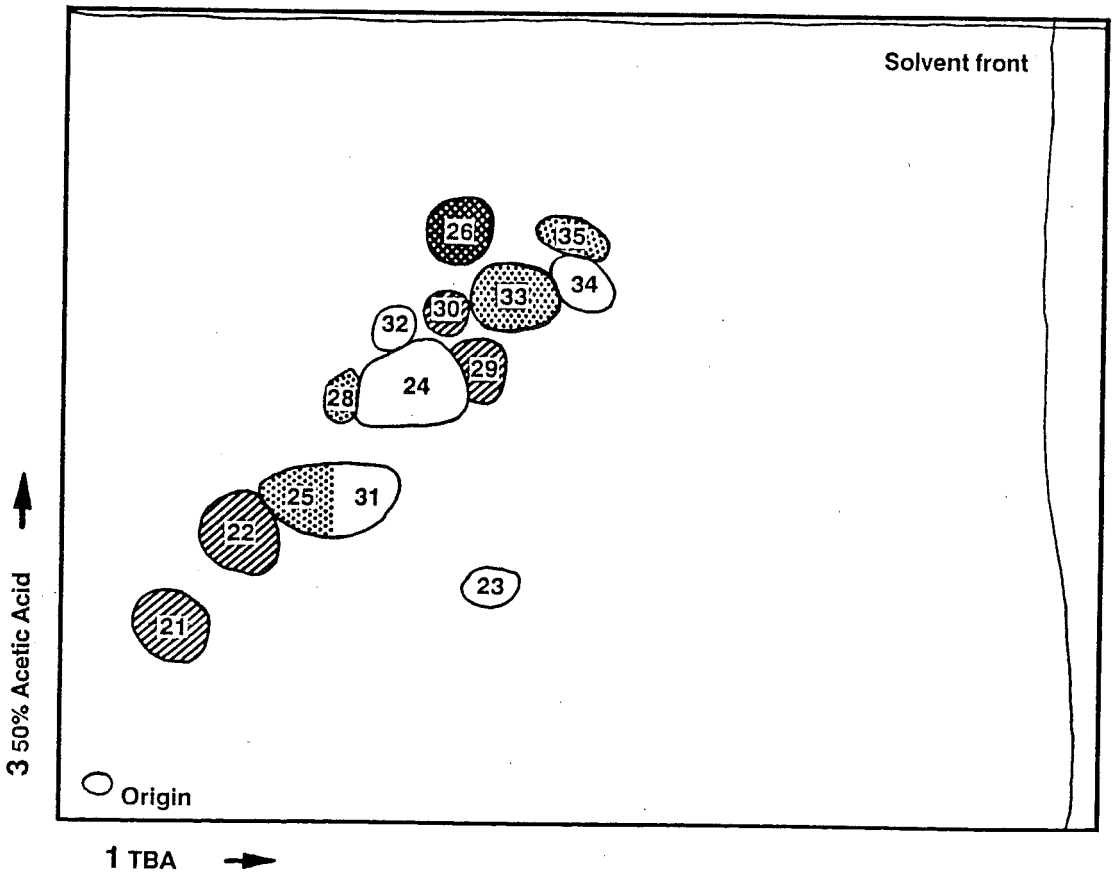


Table 3.2. Semi-quantitative distribution of leaf flavonoids in species of Gnaphaliinae.

Species	spot 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
A. keriensis	+++	+++	.	+	±	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.
A. rupestris	+++	+++	.	+	±	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.
A. subrigida	±	+++	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A. trinervis	±	+++	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A. triplinervis	.	.	.	.	.	.	.	+		±	.	.	.	.	.	.	.	.	.	.	+	+	±	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. aculeata	.	.	.	.	.	.	+	.	.	++	+++	+++	.	++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.	.
C. fulvida	.	.	.	.	.	.	±	±	.	.	?	+	.	.	.	.	+++	+++	+++	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. leptophylla	.	.	.	.	.	.	±	±	.	.	?	+	.	.	.	.	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
C. longifolia	.	.	.	.	.	.	.	.	.	+	+++	++	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. catipes	.	.	.	.	.	+++	.	.	++	.	.	.	.	.	.	.	.	.	.	.	±	++	.	.	±	.	.	+	.	.	.	.	.	.	.	.	.	.
E. meredithae	.	.	.	.	.	+++	.	.	.	.	.	.	.	+	.	.	.	.	.	.	++	±	.	.	++	.	.	.	±	.	.	.	.	.	.	.	.	.
E. planchonii	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	++	.	.	.	++	.	++	.	.	.	.	.	.	.	.	.	.
E. sinclairii	.	+	.	.	.	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	±	±	.	+	.	.	.	.	.	.	.	.	.	.	.
G. involucratum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	++	.	.	+	.	.	.	.	.	.	.	.	±	.	.	.	.
G. mackayi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	±	+	.	±	.	.	.	.	.	.	.	.	.	.
G. nitidulum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.	.	.	.	.	+	.	?	+	.	.	.	.	.	.	.	.	.	.	.	.	.
G. traversii	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ha. pulvinaris	+++	.	±	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ha. sinclairii	+++	.	?	.	.	.	.	+++	±	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. backhousii	.	.	.	.	.	.	.	±	.	.	.	±	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. bellidioides	+++	±	.	+++	+++	++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. coralloides	?	?	.	.	.	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.	.	.	.	?	.	.	.	.	.	.	.	.	.	.	.	.
He. depressum	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+++	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. dimorphum	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. filicaule	+	++	.	++	.	++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. intermedium	+++	++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. lanceolatum	.	.	.	.	.	.	.	.	.	.	?	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. obcordatum	.	.	.	.	.	.	±	.	.	.	+++	±	.	.	.	.	+	+	+	+	+++	+++	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
He. parvifolium	±	±	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+++	.	.	.	.	.	+++	.	.	.	.	.	.	.	.	.	.	.
L. grandiceps	.	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+++	+++	.	++	++	±	.	±	±	.	.	.	.	.	.	.	.	.
L. leontopodium	.	+	?	?	.	.	.	+++	+++	+	.	.	.	.	.	.	.	.	.	.	±	+	.	+++	.	±	.	.	++	±	+++	±	++	+	+	.	.	.
L. "Marlborough"	.	++	±	+++	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	++	++	.	.	.	.	.	.	.
L. "Peel"	.	++	±	+++	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	++	+	.	.	.	.	.	.	.
Pseudognaphalium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	±	.	.	±	±	.	.	±	.	.	.	.	.	.	.	.	.	.
Pterygopappus	+	.	.	.	.	.	.	++	.	.	?	+++	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	++	.
R. bryoides	.	.	.	.	.	.	.	.	.	±	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.
R. cinerea	.	±	±	.	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	±	.	.	.	.	.	.	.	.	.	.	.	.
R. eximia	++	.	++	++	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. glabra	±	.	.	.	.	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+++	+++	.	+	.	+	.	+++	.	.	.	.	.	.	.	.	.	.
R. grandiflora	.	.	.	.	.	.	.	.	.	++	.	.	.	.	.	.	.	.	.	.	+	+	.	.	++	+	.	+++	.	±	.	±	.	+++	.	.	.	.
R. hectori	.	±	.	+	++	?	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. hookeri	.	±	±	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	±	+++	.	.	+	.	.	+++	.	.	±	±	.	+	.	.	.	.
R. "L"	.	.	.	.	.	.	.	.	.	++	.	.	±	.	.	.	.	.	.	.	.	+	.	.	.	++	.	.	.	.	+	.	.	.	.	.	.	.
R. "M"	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. petriensis	.	±	.	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+++	+++	.	.	?	±	.	++	.	.	.	.	.	.	.	.	.	.
R. tenuicaulis	.	±	±	±	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	±	+++	.	.	+	.	.	+++	.	.	±	±	.	+	.	.	.	.
Genus "Z"	±	±	±	±	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	+	.	.	?	.	.	?	.	.	.	.

Key: Relative concentration, (as judged visually)
+++ = high, ++ = moderate, + = low, ± = trace, ? = inconsistent

3.3.2. Identification of compounds

Attempts were made to identify some of the compounds. Compound 1 was identified as a quercetin-3-O-glycoside by UV spectroscopy, hydrolysis and co-chromatography of the aglycone with authentic standards. Compounds 2, 3, 4 and 5 were flavonol-3-glycosides, compound 11 was a flavone-7-glycoside. The compounds 17, 18, 19 and 20 may have been surface flavonoids since they could not be detected on the chromatograms if the leaves were shaken in chloroform prior to extraction in methanol. Compounds 1, 2, 3, 4, 5 and 11 were co-chromatographed along with compounds of similar R_f values from other taxa to prove their identity. The compounds with low mobility in standard solvent systems could not be analysed because of the difficulty of extracting them from the paper.

3.3.3. Spot numbers in individual species

Three major groups of spots can be distinguished (Figure 3.4). There is the group of flavonol-3-glycosides in the centre of the 2D-PC (spots I), the spots with little mobility in standard solvent systems near the origin (spots II) and the spots with little or no mobility in 15% aq. acetic acid, but very high mobility in TBA (spots III). The New Zealand species of *Cassinia* and the Tasmanian species of *Helichrysum* have spots III in common. The species which are characterised by spots II only are *Anaphalis triplinervis, Pseudognaphalium luteoalbum, Raoulia bryoides, R. grandiflora, R.* "L", all Tasmanian *Ewartia* spp. and New Zealand *Gnaphalium* spp. except *G. traversii*. Species with only spots I are the New Zealand *Anaphalis* spp., *Helichrysum bellidioides, H. filicaule, Haastia pulvinaris* and *H. sinclairii, R. eximia* and *R.* "M", and *Pterygopappus lawrencii. Helichrysum lanceolatum* is the only species without spots belonging to those spot groups. The species not mentioned have spots I as well as spots II.

Leucogenes leontopodium has the highest number of flavonoid compounds (15), followed by *L. grandiceps* (12), *Raoulia tenuicaulis* (11), *R. hookeri* (10), *R. grandiflora*, *L.* "Marlborough" and *L.* "Peel" (8) and Genus "Z" (7). By contrast, *Helichrysum lanceolatum* and *H. dimorphum* were characterised by one spot only. The number of compounds in each group of spots and the number in total of each taxon are listed in Table 3.3.

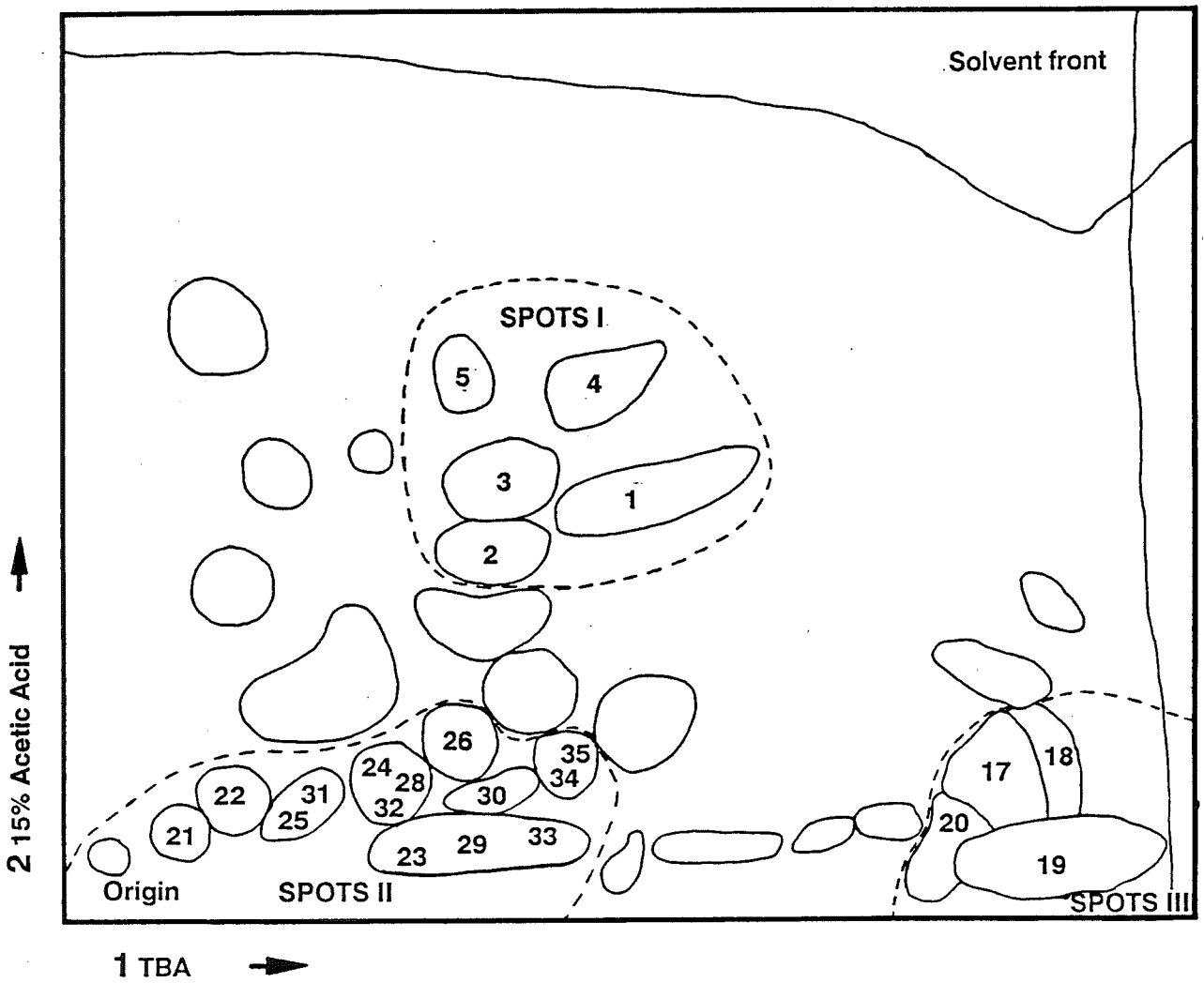


Figure 3.4. Spot groups.

Table 3.3. Spot numbers in species of Gnaphaliinae.

	Spots I	Spots II	Spots III	Total
A. keriensis	4	.	.	5
A. rupestris	4	.	.	6
A. trinervis	3	.	.	4
A. subrigida	3	.	.	3
A. triplinervis	.	4	.	6
C. aculeata	.	.	.	6
C. fulvida	.	.	4	8
C. leptophylla	.	.	4	8
C. longifolia	.	.	.	5
E. catipes	.	4	.	6
E. meredithae	.	4	.	6
E. planchonii	.	5	.	5
E. sinclairii	1	2	.	4
G. involucratum	.	5	.	5
G. mackayi	.	5	.	5
G. nitidulum	.	2	.	3
G. traversii	3	1	.	3
Ha. pulvinaris	2	.	.	3
Ha. sinclairii	2	.	.	4
He. backhousii	.	.	2	4
He. bellidioides	4	.	.	5
He. coralloides	2	1	.	1
He. depressum	1	2	.	3
He. dimorphum	1	.	.	1
He. filicaule	3	.	.	4
He. lanceolatum	.	.	.	1
He. intermedium	2	1	.	3
He. obcordatum	.	.	4	9
He. parvifolium	2	1	.	5
L. grandiceps	4	8	.	12
L. leontopodium	1	11	.	15
L. "Marlborough"	3	3	.	8
L. "Peel"	3	3	.	8
Pseudognaphalium	.	3	.	4
Pterygopappus	1	.	.	6
R. bryoides	.	1	.	3
R. cinerea	2	1	.	4
R. eximia	3	.	.	5
R. glabra	1	5	.	6
R. grandiflora	.	8	.	9
R. hectori	3	2	.	6
R. hookeri	3	7	.	10
R. "L"	.	3	.	5
R. "M"	2	.	.	2
R. petriensis	2	4	.	5
R. tenuicaulis	4	7	.	11
Genus "Z"	4	3	.	7

Spots I, II and III are the spot groups of Figure 3.4.

3.3.4. Chromatograms of individual species

The flavonoid patterns of the individual taxa will now be described considering each genus in turn.

The New Zealand species of *Anaphalis* exhibited very similar flavonoid patterns, characterised by spots 1, 2, and 4. Spot 5 and 6 were detected as a trace in *A. rupestris* and *A. keriensis*. Possibly they were below the recording limit in *A. subrigida* and *A. trinervis*. Spot 36 was characteristic for *A. rupestris* and *A. subrigida* only.

Anaphalis triplinervis, with a quite different spot pattern, had four spots close to the origin (21, 22, 23, 25) as well as spots 8 and 10.

Cassinia aculeata and *C. longifolia* had spots 10, 11, 12 and 14 in common. Spot 7 was found in *C. aculeata* only.

The chromatograms of the New Zealand *Cassinia fulvida* and *C. leptophylla* were almost identical. Both of them have, as already mentioned (see "Identification of compounds"), "surface" flavonoids and spots 12, 7 and 8.

All *Ewartia* species had spot 3 in common and, with the exception of *E. planchonii*, spot 6. Spots 21 and 22 were found in all three Tasmanian species of *Ewartia*, spot 28 only in *E. catipes* and *E. planchonii*. Specific spots of *E. catipes* were spot 9, of *E. meredithae* spots 13 and 29, of *E. planchonii* spot 26 and of *E. sinclairii* spots 2 and 24.

As already mentioned, the *Gnaphalium* spp., with the exception of *G. traversii*, lacked flavonol-3-glycosides in the centre of the 2D-PC. *G. traversii*, characterised by spots 1, 2 and 3, had no spots in common with the other species of *Gnaphalium*. The remaining *Gnaphalium* species showed spots 22 and 25. Spots 21 and 28 were found in *G. mackayi* and G. *involucratum*, while spot 26 was additional in *G. mackayi*.

The chromatograms of *Haastia pulvinaris* and *H. sinclairii* did not show many flavonoids. Both had spots 1 and 15 and possibly spot 3, a trace of which was detected consistently only in *H. pulvinaris*. Spot 15 was group specific for these two species. Spots 8 and 9 were detected in *H. sinclairii* only.

Helichrysum backhousii and *H. obcordatum* had the "surface" flavonoids mentioned in paragraph 3.3.2. Both species were characterised by spots 11 and 12. *H. obcordatum* also had spot 7 and two spots close to the origin (21, 22).

H. bellidioides and *H. filicaule* had very similar flavonoid patterns, characterised by spots 1, 2, 4 and 6, but *H. bellidioides* also had spot 5, which was detected in high concentrations.

H. depressum and *H. dimorphum* had a few flavonoids only. *H. depressum* was distinguished from *H. dimorphum* by its two spots near the origin (21, 22). They had spot 1 in common. The two leaf morphs of *H. dimorphum* were investigated separately, but they had identical flavonoid patterns.

H. coralloides, *H. parvifolium* and *H. intermedium* also had only a few flavonoids. They shared spots 1, 2 and 21. *H. coralloides* showed only traces of spot 1 and 2. One spot close to the position of spot 6 was not consistent. *H. parvifolium* showed additionally traces of spot 13 and its unique spot 27.

H. lanceolatum was characterised by spot 12 only. Spot 11 was inconsistent.

The *Leucogenes* species were characterised by large amounts of flavonoids but had only spot 2 in common. *L. grandiceps*, *L.* "Marlborough" and *L.* "Peel" shared spots 3 and 4. *L. leontopodium*, *L.* "Marlborough" and *L.* "Peel" shared spots 9, 10, 33, 34 and 35. *L. leontopodium* and *L. grandiceps* had spots 21, 22, 24, 26, 29 and 30 in common. Additional spots were for *L. grandiceps* spots 28, 35 and 44 and for *L. leontopodium* 8, 31 and 32.

Pseudognaphalium luteoalbum was mainly characterised by large amounts of bright yellow fluorescent spots. Spot 21, 24, 25 and 28 were found in traces.

Pterygopappus lawrencii has high concentrations of spot 12. The identity of spot 11 could not be determined. Other characteristic spots were 1, 8, 13 and 14.

Raoulia bryoides, *R. eximia* and *R.* "L" had only a few flavonoids, with spot 13 in common. *R.* "L" had a few spots close to the origin (22, 26 and 32). *R. bryoides* and *R.* "L" shared spot 10. *R. eximia* had no spots with low mobility in standard solvents, but had spots 1, 3 and 4 instead.

R. cinerea and *R.* "M" were marked by spots 2 and 3 only. *R. cinerea* showed traces of spot 6 and 26 as well.

R. glabra had only spot 1 in the center, but spots 21, 22, 24, 26 and 28 near the origin.

R. hectori had almost equal numbers of spots in the center (2, 4, and 5) and near the origin (21 and 22). Spot 9 was present as well.

R. hookeri and *R. tenuicaulis* were almost identical. They shared spots 2, 3, 4, 21, 22, 25, 28, 31, 32 and 34, whilst Spot 44 was found in *R. tenuicaulis* only.

R. petriensis was characterised by spots 22, 23, 26 and 28, with only traces of spot 2 and 4.

Genus "Z" had almost equal numbers of spots in the centre of the 2D-PC (1, 2, 3 and 4) and near the origin (22, 25, 28 and traces of probably 31 and 34).

3.3.5. Numerical analyses

The phenogram formed by using the simple matching coefficient and UPGMA is shown in Figure 3.5. The most evident features are as follows:

With the exception of *Haastia*, none of the 8 genera represented by more than one species form a discrete cluster. There are four major clusters: a, e, b and g.

Cluster a unites all *Leucogenes* species except *Leucogenes grandiceps* at the 0.75 level of similarity.

	OTU	Phenogram linkage levels
32	L."Marlborough"	1.0000
33	L."Peel"	0.7500
31	L.leontopodium	0.6714
7	C.fulvida	1.0000
8	C.leptophylla	0.9189
28	He.obcordatum	0.8567
20	He.backhousii	0.9189
27	He.lanceolatum	0.7858
6	C.aculeata	0.9737
9	C.longifolia	0.8289
36	R.bryoides	0.8947
43	R."L"	0.8041
35	Pterygopappus	0.7404
42	R.hookeri	0.9737
46	R.tenuicaulis	0.9028
47	Genus "Z"	0.8036
30	L.grandiceps	0.7587
12	E.planchonii	1.0000
15	G.mackayi	0.9474
14	G.involucratum	0.9279
45	R.petriensis	0.9070
10	E.catipes	0.8913
39	R.glabra	0.8581
40	R.grandiflora	0.8444
5	A.triplinervis	0.8684
16	G.nitidulum	0.8553
11	E.meredithae	0.7923
13	E.sinclairii	0.8947
34	Pseudognaphalium	0.8363
18	Ha.pulvinaris	0.9459
19	Ha.sinclairii	0.8930
38	R.eximia	0.8705
3	A.subrigida	0.9737
4	A.trinervis	0.9408
1	A.keriensis	1.0000
21	He.bellidioides	0.9737
25	He.filicaule	0.9649
2	A.rupestris	0.8866
17	G.traversii	0.9730
44	R."M"	0.9331
37	R.cinerea	0.8958
22	He.coralloides	1.0000
26	He.intermedium	0.9594
24	He.dimorphum	0.9542
23	He.depressum	0.9192
29	He.parvifolium	0.8295
41	R.hectori	0.0000

Cophenetic correlation = 0.705

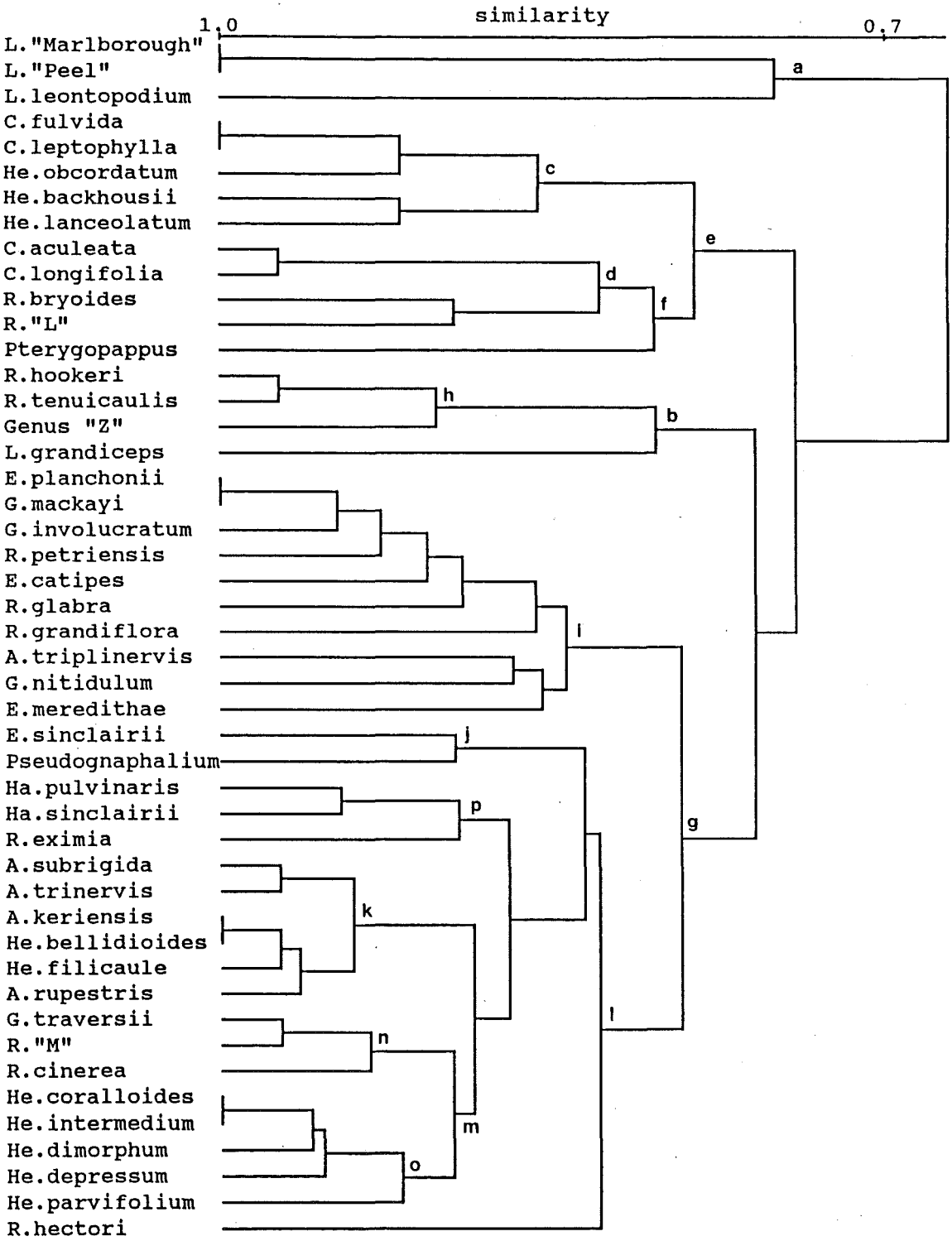


Figure 3.5. UPGMA phenogram from flavonoid data with simple matching coefficient.

Forming cluster b, *Leucogenes grandiceps* joins cluster h at 0.8. Cluster h has two very distinct components, the first comprising *Raoulia hookeri* and *R. tenuicaulis*, which join at 0.97, and the second Genus "Z", which joins the first mentioned group at 0.90.

Cluster e unites cluster c and f at the similarity level of 0.74. Cluster c comprises the New Zealand species of *Cassinia*, the Tasmanian species of *Helichrysum* and *Helichrysum lanceolatum* at the similarity level of 0.78. The Tasmanian species of *Cassinia* join each other at 0.97 and are associated at the similarity level of 0.82 with the pulvinate species of *Raoulia*, except *R. eximia*, to form cluster d. Cluster d and *Pterygopappus lawrencii* are united at 0.80 to form cluster f.

All the remaining species form cluster g, which splits into cluster i and l at the level of 0.79.

Cluster l contains the discrete cluster k which unites the New Zealand species of *Anaphalis* with *Helichrysum bellidioides* and *H. filicaule* at the high similarity level of 0.94. Cluster k is joined by cluster m at 0.89. Cluster m is formed by cluster n consisting of *Raoulia* "M", *R. cinerea* and *Gnaphalium traversii* and cluster o consisting of *Helichrysum coralloides*, *H. intermedium*, *H. dimorphum*, *H. depressum* and *H. parvifolium*. Cluster p combines *Haastia pulvinaris* and *H. sinclairii* (0.94) with *Raoulia eximia* at 0.90. Cluster j unites *E. sinclairii* and *Pseudognaphalium luteoalbum* at 0.89. *Raoulia hectori* remains isolated until it joins the combined clusters k, m, p and j at 0.83 to complete cluster l.

Cluster i includes all *Gnaphalium* species except *Gnaphalium traversii*, the Tasmanian *Ewartia* species, *Anaphalis triplinervis*, *Raoulia glabra, R. grandiflora* and *R. petriensis*. There is no distinct cluster within cluster i. *E. planchonii* and *G. mackayi* pair at 1.00 similarity and are joined by *G. involucratum* at 0.95 similarity, *R. petriensis* at 0.93, *E. catipes* at 0.91, *R. glabra* at 0.90 and *R. grandiflora* at 0.86. *A. triplinervis* and *G. nitidulum* pair at 0.87 similarity and are joined by *E. meredithae* at 0.86 before they join the rest of cluster i at 0.84.

The cophenetic correlation coefficient was 0.705. The similarity matrix is given in Appendix 6.

Figure 3.6 shows the phenogram formed by using Jaccard's coefficient and UPGMA.

The similarity levels are very low in comparison to those in Figure 3.5 and the major clusters are not as obvious. There are major changes at very low similarity levels where distortion is to be expected (Ward, 1981).

Some OTUs show substantially different closest relationships. For example *Helichrysum lanceolatum* is now isolated from cluster c. *Raoulia hectori* is joining cluster b at a low similarity level (0.5). *Helichrysum dimorphum*, instead of being associated with *H. coralloides* and *H. intermedium*, is very isolated as well. Cluster b, *Raoulia tenuicaulis, R. hookeri*, Genus "Z" and *Leucogenes grandiceps*, is included in cluster l. *Helichrysum depressum* pairs with *Raoulia glabra* within cluster l. *Raoulia eximia* is associated not with *Haastia*, but with the *Anaphalis* group k. *Raoulia bryoides* and *R.* "L" do not have affinities to any other group and are not even very similar to each other.

The cophenetic correlation coefficient was 0.844. The similarity matrix is given in Appendix 6.

Figure 3.7 shows the phenogram formed by using the simple matching coefficient and single linkage clustering.

The most isolated species is *Leucogenes leontopodium* which joins with all the other species at 0.75. Identical with each other but also quite isolated from all else are *L.* "Marlborough" and *L.* "Peel", which are associated with the rest of the species at 0.84. *Anaphalis triplinervis*, *Leucogenes grandiceps* and *Pterygopappus lawrencii* are separated from the big cluster a until 0.87. *Raoulia hookeri* and *R. tenuicaulis* again form a distinct cluster with Genus "Z" and join the remaining species of cluster a at 0.89. *R. hectori* joins separately at almost the same level. *Raoulia bryoides, R.* "L", *R. grandiflora, Gnaphalium nitidulum* and *Ewartia meredithae* all join cluster b at 0.89. *Ewartia sinclairii* and *Pseudognaphalium luteoalbum* join at 0.914, while the other isolated species *Helichrysum obcordatum* and *H. backhousii*, the paired *Cassinia fulvida* and *C. leptophylla* and the paired *Cassinia aculeata* and *C. longifolia* are united with the clusters c and d at the level 0.918. Cluster d is formed by *Ewartia planchonii, Gnaphalium mackayi, G. involucratum, Raoulia petriensis* and *E. catipes*. In cluster c are three distinct clusters combined with some isolated OTUs. One cluster joins

	OTU	Phenogram linkage levels
32	L."Marlborough"	1.0000
33	L."Peel"	0.4000
31	L.leontopodium	0.1422
13	E.sinclairii	0.3333
34	Pseudognaphalium	0.1764
5	A.triplinervis	0.3333
11	E.meredithae	0.2858
23	He.depressum	0.6000
39	R.glabra	0.3943
12	E.planchonii	1.0000
15	G.mackayi	0.6667
45	R.petriensis	0.5873
14	G.involucratum	0.5223
10	E.catipes	0.4861
40	R.grandiflora	0.3329
42	R.hookeri	0.9091
46	R.tenuicaulis	0.6333
47	Genus "Z"	0.4872
30	L.grandiceps	0.3587
41	R.hectori	0.2268
16	G.nitidulum	0.1156
36	R.bryoides	0.3333
43	R."L"	0.1006
18	Ha.pulvinaris	0.5000
19	Ha.sinclairii	0.2917
24	He.dimorphum	0.1690
22	He.coralloides	1.0000
26	He.intermedium	0.4667
29	He.parvifolium	0.1958
17	G.traversii	0.6667
44	R."M"	0.4500
37	R.cinerea	0.2660
1	A.keriensis	1.0000
21	He.bellidioides	0.8333
2	A.rupestris	0.7556
25	He.filicaule	0.5896
3	A.subrigida	0.7500
4	A.trinervis	0.3147
38	R.eximia	0.0272
7	C.fulvida	1.0000
8	C.leptophylla	0.6667
28	He.obcordatum	0.4810
20	He.backhousii	0.1628
6	C.aculeata	0.8333
9	C.longifolia	0.2361
35	Pterygopappus	0.2056
27	He.lanceolatum	0.0000

Cophenetic correlation = 0.844

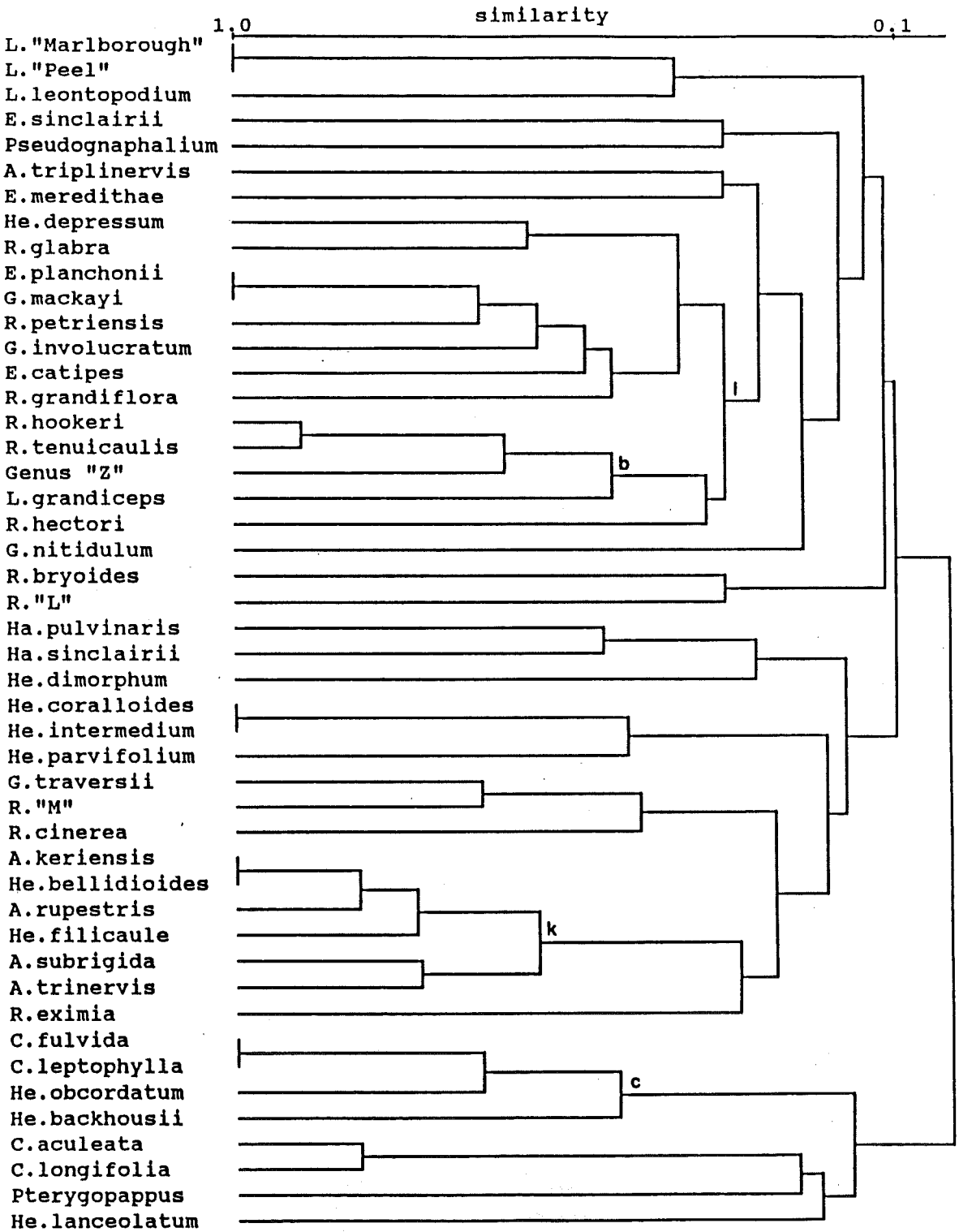


Figure 3.6. UPGMA phenogram from flavonoid data with Jaccard's coefficient.

	OTU	Phenogram linkage levels
32	L."Marlborough"	1.0000
33	L."Peel"	0.8421
42	R.hookeri	0.9737
46	R.tenuicaulis	0.9167
47	Genus "Z"	0.8889
36	R.bryoides	0.8947
43	R."L"	0.8947
6	C.aculeata	0.9737
9	C.longifolia	0.9189
7	C.fulvida	1.0000
8	C.leptophylla	0.9189
28	He.obcordatum	0.9189
20	He.backhousii	0.9189
17	G.traversii	0.9730
44	R."M"	0.9474
37	R.cinerea	0.9459
1	A.keriensis	1.0000
21	He.bellidioides	0.9737
25	He.filicaule	0.9737
3	A.subrigida	0.9737
4	A.trinervis	0.9737
2	A.rupestris	0.9474
22	He.coralloides	1.0000
26	He.intermedium	0.9714
24	He.dimorphum	0.9706
23	He.depressum	0.9474
29	He.parvifolium	0.9474
18	Ha.pulvinaris	0.9459
27	He.lanceolatum	0.9459
19	Ha.sinclairii	0.9444
39	R.glabra	0.9211
38	R.eximia	0.9189
12	E.planchonii	1.0000
15	G.mackayi	0.9474
14	G.involucratum	0.9459
45	R.petriensis	0.9211
10	E.catipes	0.9143
34	Pseudognaphalium	0.9143
13	E.sinclairii	0.8947
40	R.grandiflora	0.8947
16	G.nitidulum	0.8947
11	E.meredithae	0.8919
41	R.hectori	0.8684
5	A.triplinervis	0.8649
35	Pterygopappus	0.8649
30	L.grandiceps	0.7500
31	L.leontopodium	0.0000

Cophenetic correlation = 0.629

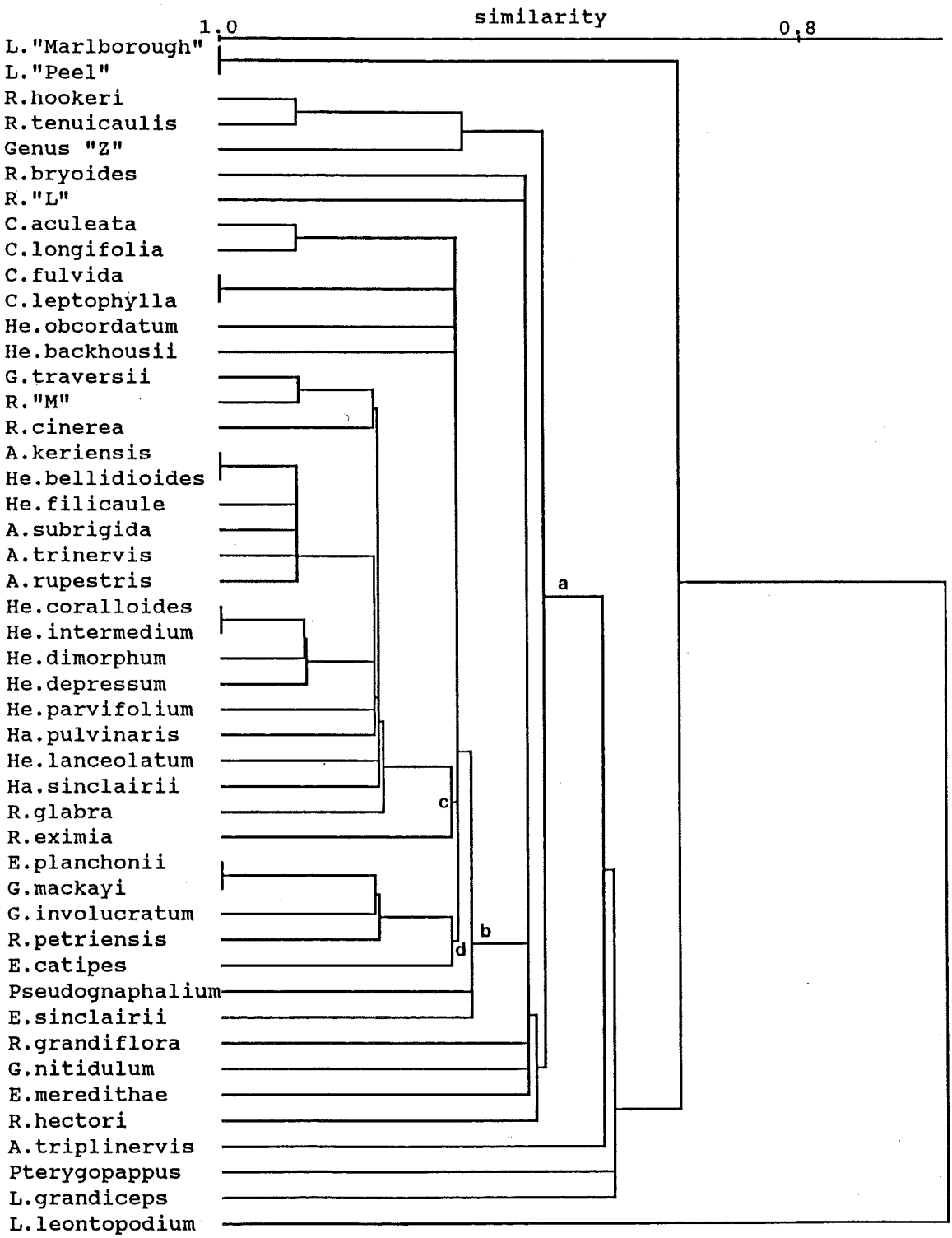


Figure 3.7. Single linkage phenogram from flavonoid data with simple matching coefficient.

Gnaphalium traversii, *Raoulia* "M" and *R. cinerea*, the second cluster is formed by *Helichrysum coralloides*, *H. intermedium*, *H. dimorphum* and *H. depressum* and the third cluster includes the New Zealand *Anaphalis* species with *Helichrysum bellidioides* and *H. filicaule*.

The cophenetic correlation coefficient was 0.629. The similarity matrix is given in Appendix 6.

Figure 3.8 shows the phenogram formed by using Jaccard's coefficient and single linkage clustering. Similarities are very low and discrete clusters are few.

The cluster of *Cassinia fulvida*, *C. leptophylla*, *Helichrysum backhousii* and *H. obcordatum* is formed at the similarity level of 0.6. *Cassinia aculeata* and *C. longifolia* join at 0.83. *Helichrysum coralloides* and *H. intermedium* form a cluster with *H. parvifolium* at 0.6. *Gnaphalium traversii* and *Raoulia* "M" cluster at 0.7. The New Zealand *Anaphalis* species in this phenogram again form a very distinct cluster with *Helichrysum bellidiodes* and *H. filicaule*. *Ewartia planchonii* and *Gnaphalium mackayi* are associated once more with *Raoulia petriensis* and *G. involucratum*. The cluster of *Raoulia hookeri* and *R. tenuicaulis* with Genus "Z" remains quite distinct, but the two *Leucogenes* species *L.* "Marlborough" and *L.* "Peel" are isolated from *L. leontopodium*. All the remaining species are very isolated or form clusters at a low similarity level.

The cophenetic correlation coefficient was 0.683. The similarity matrix is given in Appendix 6.

Comparison of the four phenograms:

Some clusters are very consistent in all phenograms under consideration. The New Zealand *Anaphalis* species are always very closely associated with *Helichrysum bellidioides* and *H. filicaule*. A very consistent cluster is formed by *Raoulia tenuicaulis*, *R. hookeri* and Genus "Z". *Ewartia planchonii*, *Gnaphalium mackayi*, *G. involucratum* and *R. petriensis* are closely linked in the four phenograms, while *Ewartia catipes* and *Raoulia grandiflora* are usually associated with them. *R. cinerea* is associated with the paired *Gnaphalium traversii* and *Raoulia* "M".

	OTU	Phenogram linkage levels
32	L."Marlborough"	1.0000
33	L."Peel"	0.4000
1	A.keriensis	1.0000
21	He.bellidioides	0.8333
2	A.rupestris	0.8000
25	He.filicaule	0.7500
3	A.subrigida	0.7500
4	A.trinervis	0.5000
42	R.hookeri	0.9091
46	R.tenuicaulis	0.6667
47	Genus "Z"	0.5333
12	E.planchonii	1.0000
15	G.mackayi	0.6667
45	R.petriensis	0.6667
14	G.involucratum	0.5714
23	He.depressum	0.6000
39	R.glabra	0.5714
10	E.catipes	0.5556
40	R.grandiflora	0.5455
30	L.grandiceps	0.5000
41	R.hectori	0.5000
22	He.coralloides	1.0000
26	He.intermedium	0.6000
29	He.parvifolium	0.5000
17	G.traversii	0.6667
44	R."M"	0.5000
37	R.cinerea	0.5000
18	Ha.pulvinaris	0.5000
19	Ha.sinclairii	0.5000
11	E.meredithae	0.4118
31	L.leontopodium	0.4000
43	R."L"	0.4000
38	R.eximia	0.3750
5	A.triplinervis	0.3333
36	R.bryoides	0.3333
24	He.dimorphum	0.3333
16	G.nitidulum	0.3333
13	E.sinclairii	0.3333
34	Pseudognaphalium	0.2500
7	C.fulvida	1.0000
8	C.leptophylla	0.6667
28	He.obcordatum	0.5714
20	He.backhousii	0.2500
35	Pterygopappus	0.2500
27	He.lanceolatum	0.2500
6	C.aculeata	0.8333
9	C.longifolia	0.0000

Cophenetic correlation = 0.683

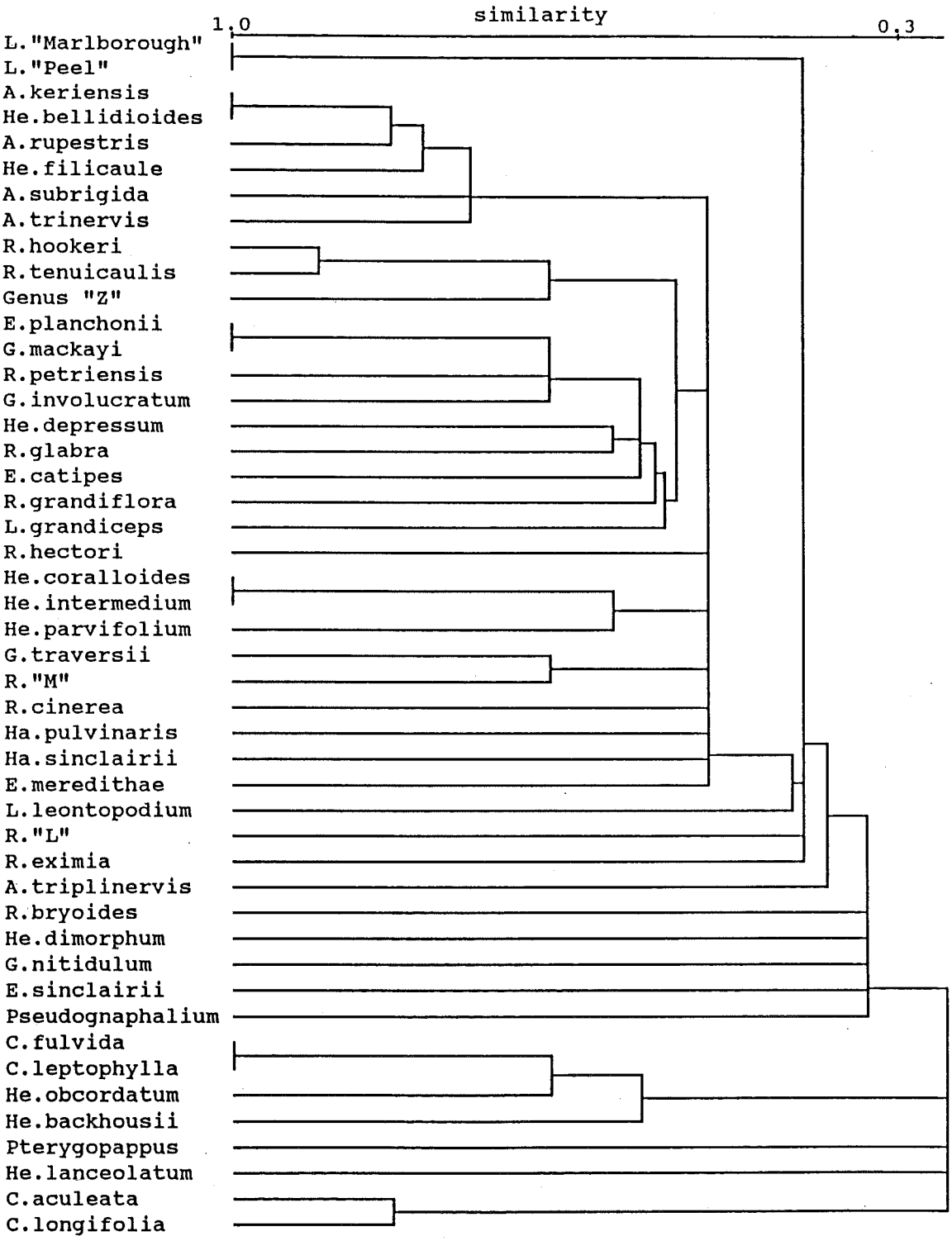


Figure 3.8. Single linkage phenogram from flavonoid data with Jaccard's coefficient.

The Tasmanian species of *Cassinia* form a cluster on their own while the New Zealand species of *Cassinia* are usually associated with the Tasmanian species of *Helichrysum* sect. *Ozothamnus*.

Some clusters are less consistent. The paired *H. coralloides* and *H. intermedium* have a high similarity level with *H. parvifolium*, but *H. dimorphum* and *H. depressum* are associated with the latter species only when using the simple matching coefficient. The phenograms obtained by using Jaccard's coefficient link *H. depressum* with *R. glabra*, while the position of *H. dimorphum* varies. *Leucogenes leontopodium* forms a cluster with the paired *L.* "Marlborough" and *L.* "Peel" in the UPGMA phenograms but it is isolated in the two single linkage phenograms.

The remaining species have changing affinities which are in some cases correlated with the clustering method. Thus in the UPGMA phenograms *Leucogenes grandiceps* joins the cluster of *Raoulia hookeri, R. tenuicaulis* and Genus "Z", but it has different links in single linkage phenograms. *Anaphalis triplinervis* is isolated in the single linkage phenograms but shows some affinities with *Ewartia meredithae* and in one case with *Gnaphalium nitidulum* in the UPGMA phenogram. *Ewartia sinclairii* and *Pseudognaphalium luteoalbum* are paired in the UPGMA phenograms but more loosely associated in the single linkage phenograms. The same is true of *Raoulia bryoides* and *R.* "L" and also of the two species of *Haastia. Pterygopappus lawrencii*, *Raoulia eximia*, *R. hectori* and *Helichrysum lanceolatum* have different positions in all four phenograms.

Considering the genera, only *Haastia* forms a distinct cluster. The New Zealand species of *Anaphalis* are quite distant from *Anaphalis triplinervis*. *Leucogenes grandiceps* is not linked with the other *Leucogenes species* and even those do not from consistent clusters in all four phenograms. *Helichrysum* is represented by 10 species in two sections. The species of *Helichrysum* sect. *Xerochlaena* cluster with the New Zealand *Anaphalis* species. Concerning *Helichrysum* sect. *Ozothamnus*, only *H. intermedium, H. parvifolium* and *H. coralloides* cluster together consistently. *H. dimorphum* is either remotely associated with this group or has different affinities, while *H. depressum* either joins this group or is united with *Raoulia glabra*, and *H. lanceolatum* has changing positions. The Tasmanian species of this section cluster with the New Zealand species of *Cassinia*. The Tasmanian *Cassinia* species form a cluster on their own.

The Tasmanian *Ewartia* species are not very closely associated, but are clearly separated from the New Zealand species. In *Gnaphalium, G. involucratum* and *G. mackayi* are linked together, while *G. nitidulum* is remotely associated with this group in only two of the phenograms and *G. traversii* has affinities with other taxa. The only *Raoulia* species joined are *R. hookeri* with *R. tenuicaulis* and, in two of the four phenograms, *R. bryoides* with *R.* "L". The other species are isolated or form links to other genera.

3.4. Discussion

The use of spot pattern characters

As pointed out by Harborne and Turner (1984), the use of spot data of two-dimensional chromatograms without identification of individual consituents can lead to a misuse of these data. One problem in using spot data in taxonomic studies is that the same position on a chromatogram is not necessarily occupied by the same compound (Harborne, 1975). However, Harborne also comments that this is less likely when comparing chromatograms of very closely related taxa. Another problem is the danger of over-estimating the number of constituents really present (Harborne, 1975b). For example, labile flavonoid glycosides can be broken down to the aglycone and thus give two or more spots on a chromatogram. This could lead to over-estimation of the taxonomic distance. Likewise, following Adam's (1974) argument, the same problem could occur with morphological characters since most of the time their mode of inheritance or morphogenetic path is not known.

It was not possible in this preliminary study to identify all flavonoids, but the minimal requirements for a chemotaxonomic analysis (Harborne, 1975), that is identifying some of the major compounds and testing the homogeneity of the other compounds by co-chromatographing them in several solvent systems, were accomplished. Only the spots with low mobility in standard solvent systems could not be tested because of the difficulty of eluting them from the paper.

Analysis of the chemical data

From the early days of chemotaxonomy spot-data have been treated phenetically (e.g., Kaltsikes and Dedio, 1970; Heubl and Vogt, 1985). There has been much discussion about the use and misuse of spot data and their numerical analysis (e.g., Weimark, 1972, 1974; Crawford *et al.*, 1974; Adams, 1974). Nowadays most chemotaxonomic publications include detailed flavonoid analyses, thus avoiding some of the above mentioned problems, but still only a few chemotaxonomists undertake phenetic or phylogenetic analysis with their flavonoid data.

Cladistic treatments (for example, as published by Williams and Garnock-Jones, 1986 or Park, 1987) are quite rare, though flavonoid data fulfill fully the requirements of this type of analysis (Harborne and Turner, 1984). A cladistic analysis was not undertaken in this study. Before proposing an evolutionary hypothesis, all flavonoids should be identified and more specimens of each taxon should be examined, so the present study can only be regarded as a preliminary analysis.

Nevertheless, the data of this study were regarded as adequate for a phenetic analysis subject to the following condition. In agreement with Jensen and Nielsen (1985), in their introduction to chemical characters in the monograph of Dahlgren *et al.* on the monocotyledons, chemical characters should be used only in combination with other characters. This condition is even more important in the present study since it does not contain full flavonoid analysis. A phenetic analysis of the flavonoids seemed therefore to be most appropriate since it allowed direct comparison with Ward's (1981) phenetic analysis of the Gnaphaliinae based on 165 morphological characters.

Numerical analysis

The chemical characters were treated in the numerical analyses as "present" or "absent", or in the case of uncertain character states, as missing values. Thus the criticism of Harborne and Turner (1984) that numerical analysis does not allow missing data, does not apply.

Two similarity coefficients were used for the following reasons. The simple matching coefficient gives equal weight to shared present and absent characters and this seems to be appropriate in the present study. It has been used in several other flavonoid studies (e.g., Kaltsikes and Dedio, 1970). Jaccard's coefficient, which ignores shared absences, was used because the absence of a character may depend partly on the sensitivity of the method of detection used. Therefore only compounds verified as present were included in the calculation. The second effect of using Jaccard's coefficient is that it counts only shared presences as similarities and these may be more taxonomically significant than shared absences. Ward (1981) states that whether to include or exclude negative matches in an analysis depends on the significance of the "absence" state as judged by the taxonomist.

Two different clustering methods were used. UPGMA clustering was used since generally it gives the least amount of distortion of a similarity matrix (Rohlf, 1970; Sneath and Sokal, 1973). UPGMA clustering is widely used in taxonomy and according to Ward (1981) provides the best basis for a classification. Single linkage clustering was used as an additional clustering method only because it provides information on closest phenetic relationships. It tends to produce phenograms with chaining and poor hierarchical structure (Ward, op. cit.).

All four phenograms were compared with one another since arrangements which are consistent in all phenograms are more likely to be useful for any taxonomic treatment than those which differ from one phenogram to another (Ward, op. cit.).

Taxonomic implications of the flavonoid analysis

Results of the present study partially support previous hypotheses concerning taxonomic relationships within the Gnaphaliinae but also propose relationships not recognised previously.

The New Zealand species of *Anaphalis*, treated by Allan (1961) in *Gnaphalium*, were transferred by Webb *et al.*(1987) into *Anaphalis*. Webb *et al.* agreed with Drury (1970) who proposed this transfer based on the subdioecy of these species. The flavonoid pattern suggests also that *Anaphalis keriensis, A. trinervis, A. rupestris* and *A. subrigida* have to be separated from *Gnaphalium*. The comparison with the flavonoid pattern of *Anaphalis triplinervis* does not underline similarities and therefore casts some doubt on the position of the New Zealand *Anaphalis* species within *Anaphalis*. The species of *Helichrysum* sect. *Xerochlaena*, *H. bellidioides* and *H. filicaule*, are associated in the phenograms of the flavonoid data with the New Zealand *Anaphalis* species. Drury (1971) noted that *Helichrysum bellidioides* is probably an anaphalioid cudweed, but it was not transferred by Webb *et al.* into *Anaphalis* because it hybridises freely with other species currently treated in New Zealand within *Helichrysum* and their affinities are yet to be determined. Thus the flavonoid pattern of *Helichrysum bellidioides* supports close affinities with the New Zealand species of *Anaphalis*. *Helichrysum filicaule* is associated in this analysis with the anaphalioid group and not, in comparison to Ward's treatment (1981), remotely with *Raoulia cinerea*. According to the flavonoid pattern, *Helichrysum* sect. *Xerochlaena* is a distinct entity and it should not be treated within *Helichrysum* but instead within the New Zealand species of *Anaphalis*.

Anaphalis triplinervis, having its origin in the Himalayas and included into this study to compare the New Zealand *Anaphalis* species with a "true" *Anaphalis*, has no close affinities to any of the other taxa. Its closest similarities are with the *Gnaphalium-Ewartia* group.

Cassinia and the Tasmanian species of *Helichrysum* sect. *Ozothamnus* are quite different from all the other species investigated. It is noteworthy that the New Zealand species of *Cassinia* cluster with the Tasmanian *Helichrysum* species while the Tasmanian species of *Cassinia* form a cluster on their own. Hooker (1864) remarked that *Cassinia fulvida* might be more correctly placed in *Ozothamnus*, and that *C. vauvilliersii* was scarcely distinguishable from a true *Ozothamnus* of Tasmania, *O. cuneifolius*. The flavonoid pattern definitely supports this opinion.

It is of interest that the Tasmanian species of *Helichrysum* sect. *Ozothamnus* show no affinities in their flavonoid pattern to the New Zealand species of this section, thus supporting Ward's proposal (pers. comm.) to separate the New Zealand group from *Helichrysum*.

The flavonoid patterns indicate that the New Zealand species of *Helichrysum* sect. *Ozothamnus* are not a homogeneous group. Only *Helichrysum coralloides, H. parvifolium* and *H. intermedium* are consistently associated.

Helichrysum dimorphum, though quite isolated, has its closest affinities to this latter group. It was hoped that the flavonoid studies would help to clear up the mystery of this strange species, the origin of which is still not known. Wall (1920) proposed that it might be a hybrid of *Helichrysum filicaule* and *Helichrysum depressum*. Flavonoids are known to be useful in determining the origin of hybrids (Webby *et al.* 1987; Williams *et al.* 1983), but unfortunately the species concerned possess only a few flavonoids. All three species have compound 1. *H. depressum* is characterised in addition by spots 21 and 22, and *H. filicaule* by spots 2, 4 and 5, while *H. dimorphum* has no additional spots. This means that *H. dimorphum* has less spots than the proposed parents. However, this information is not sufficient to reject Wall's proposal. More detailed flavonoid analysis might be useful since there may be considerable quantitative alterations, as reported for other hybrids by Harborne (1967). Occasionally parental compounds may be missing or, rarely, additional compounds may appear (Alston *et al.*, 1965; Webby *et al.*, 1987).

Helichrysum depressum is quite isolated from the other *Helichrysum* species not only in Ward's phenograms (1981) but also in this analysis of the flavonoid patterns. Some of Ward's phenograms indicate remote affinities with *Raoulia* subg. *Raoulia*. Its closest relationship in the flavonoid study is with *Raoulia glabra* and also with *Helichrysum coralloides-H. intermedium.*

The affinities of *Helichrysum lanceolatum* are unclear because of the lack of flavonoid spots. Its closest relationships are within the cluster of *Cassinia* and the Tasmanian *Helichrysum* species. Thus the flavonoid pattern does not reveal any links to other groups, but underlines the uncertain position of this taxon.

The two species of *Haastia* cluster with one another. A remote affinity of *Haastia* to *Raoulia eximia* or *Helichrysum dimorphum* can be recognised . Spot 15 is unique to *Haastia*.

This analysis supports Merxmüller *et al.* (1977) in suggesting the inclusion of *Haastia pulvinaris* in the Gnaphaliinae, since the two *Haastia* species are not more isolated than other examined groups of the Gnaphaliinae. The flavonoid pattern does not suggest, however, that those two species are heterogeneric, as proposed by Merxmüller *et al.* (op. cit.). The two species are more similar to one another than to any other taxon. The relationship of *Haastia pulvinaris* with *Pterygopappus lawrencii* suggested by Merxmüller *et al.* (op. cit.) finds no support in the flavonoid pattern. *Pterygopappus* stands very isolated without any consistent affinities.

Pseudognaphalium luteoalbum, included in this genus by Hilliard and Burtt (1981), supports the separation from *Gnaphalium* by having a very distinct pattern on the 2D-PCs without close similarities. The large number of bright yellow fluorescent compounds especially underlines its isolated position. The similarity indices show remote affinities to *Ewartia sinclairii*, which are indicated as well in Ward's (op. cit.) phenograms.

The genus *Ewartia*, represented in this study by *Ewartia sinclairii*, endemic to New Zealand, and by three species endemic to Tasmania, does not form a unique group, thus supporting Ward's studies (1981). The flavonoid pattern of *Ewartia sinclairii* is quite different from that of the Australian species.

The Tasmanian species of *Ewartia* have high similarity to one another and also to the species of *Gnaphalium* and to *Raoulia petriensis*. *Ewartia planchonii* and *Gnaphalium mackayi* have an identical 2D PC pattern with only quantitative differences, thus enhancing the possibility of an even closer relationship of the Tasmanian *Ewartia* species with *Gnaphalium* than is suggested by Ward (1981). *Ewartia catipes* and *E. planchonii* are closer to one another than to *E. meredithae*, indicated as well by Ward (op. cit.). The paired *Ewartia planchonii* and *Gnaphalium mackayi* are most similar to *Gnaphalium involucratum* and *Raoulia petriensis*, followed by *Ewartia catipes*. The relationship to *Gnaphalium* is obvious.

The anaphalioid group of *Gnaphalium* was transferred to *Anaphalis* by Webb (1987) and New Zealand's only member of the achyroclinoid group, *Gnaphalium luteo-album*, was removed to *Pseudognaphalium* by Hilliard and Burtt (1981). All the remaining New Zealand species of

Gnaphalium belong to sect. *Euchiton*. However, from the flavonoid point of view the four species examined, *Gnaphalium involucratum* , *G. mackayi, G. nitidulum* and *G. traversii* do not form a coherent group. *G. traversii* is distinguished from the other species by having spots 1, 2 and 3, and by the lack of spots with low mobility in the standard solvent systems. In all flavonoid phenograms it forms a cluster with *Raoulia* "M" and *Raoulia cinerea*. Ward (1981) mentions distant associations of *Raoulia* "M" with *Gnaphalium*, but only *Gnaphalium mackayi* and *G. nitidulum* were included in her studies. The phenograms based on the flavonoid data indicate relationships of the cluster of *Gnaphalium traversii*, *Raoulia cinerea* and *Raoulia* "M" with several other groupings but not with the other *Gnaphalium* species. Since the flavonoids are the first characters which split the species of *Gnaphalium* into two groups, this split has to be evaluated carefully. On one side it suggests more detailed flavonoid studies with the aim of identifying the flavonoid compounds under consideration, on the other side, this split requires a thorough review of morphological characters or the investigation of characters not yet looked at.

Not even *Leucogenes* seems to be homogeneous. *Leucogenes*, represented by two described species, *L. grandiceps* and *L. leontopodium*, and two undescribed species *L.* "Marlborough" and *L.* "Peel", splits into two entities. *L. leontopodium*, *L.* "Marlborough" and *L.* "Peel" form an isolated group while *L. grandiceps* has its closest similarity to *Raoulia petriensis*. *L.* "Marlborough" and *L.* "Peel" have almost identical flavonoid patterns, rather different from that of *Leucogenes leontopodium*. This difference might be due to the fact that the specimens of *L.* "Marlborough" and *L.* "Peel" examined were cultivated in glasshouses, while the specimen of *L. leontopodium* was collected in the field. Loss of flavonoids of cultivated plants has been reported by Anderson (1987) who reported studies of *Solanum* showing that extracts from the same accession from original field collections and from garden-grown plants exhibited a greater array of flavonols than glasshouse-grown plants. However, since the present study did not aim to investigate the species differentiation of the *Leucogenes leontopodium* complex, a study in process by Brian Molloy (pers. comm.), but to investigate the relationship of the different genera of the Gnaphaliinae, the information obtained about the two groups within the *Leucogenes leontopodium* complex is interesting but irrelevant to the present study.

Genus "Z", an undescribed genus (Ward, pers.comm.), was suggested as comparable to *Haastia sinclairii* or *Leucogenes grandiceps* or possibly of hybrid origin (Allan, 1961). The relationship to either *Leucogenes* or *Haastia* finds no support in the flavonoid pattern. The species of *Haastia* have no spots with low mobility in standard solvent systems, whilst Genus "Z" has quite a few of them, but it has no spot 15 which is characteristic of *Haastia*. In the flavonoid phenograms, the position of Genus "Z" lies between *Raoulia* subg. *Raoulia* and *Leucogenes grandiceps*. It must be noted that two spots with low mobility had to be treated as uncertain since their identity could not be proved.

In the flavonoid studies *Raoulia* is not a single group. Ward (1981) states that the genus as presently constituted contains two very different and clearly demarcated species groups, as well as a number of species of uncertain affinities.

According to Ward, the species of *Raoulia* subg. *Raoulia* form a coherent if internally variable group, provided that *R. cinerea* and *R.* "M" are removed. In Ward's and also in the present study, *R. hookeri* and *R. tenuicaulis* form a consistent group, clearly belonging to *Raoulia* subg. *Raoulia*. *R. glabra* has in Ward's study an isolated position within *Raoulia* subg. *Raoulia*. The flavonoid pattern suggests even an exclusion from *Raoulia* and a possible alliance with *Helichrysum depressum*. Not only Ward's thesis but also the present study suggest that *R. cinerea* and *R.* "M" should be excluded from *Raoulia*. In contrast to Ward's results which suggest slight affinities of *R. cinerea* with *Helichrysum filicaule*, *R. cinerea* and *R.* "M" are very similar to one another in their flavonoid patterns, and both are very similar to *Gnaphalium traversii*. A possible alliance of *R.* "M" with part of *Gnaphalium* was suggested also by Ward.

R. petriensis is the only member of subgenus *Mistura*. Neither Ward's thesis nor the present study strongly support inclusion in *Raoulia*, but otherwise the results differ in that Ward's studies suggest weak links to *Leucogenes* and *Ewartia*, while the flavonoid study indicates strong links to part of *Gnaphalium* and *Ewartia*.

R. grandiflora and *R. hectori* belong to the non-pulvinate species of *Raoulia* subg. *Psychrophyton*. *R. grandiflora* is in Ward's study as related to *Leucogenes* as to other non-pulvinate species (*R. subulata* and *R. hectori*) of *Raoulia* subg. *Psychrophyton*. In the present

study it is related to neither of them. Affinities to the group containing part of *Gnaphalium* and *Ewartia* and *R. petriensis* are shown.

R. hectori, in Ward's study associated with the species of *Raoulia* subg. *Psychrophyton* and *Leucogenes*, is in the present study quite isolated with one remote link to part of *Raoulia* subg. *Raoulia*, *Leucogenes grandiceps* and Genus "Z".

According to Ward, the pulvinate species of *Raoulia* subg. *Psychrophyton* form a coherent, uniform group, quite separated form *Raoulia* subg. *Raoulia*. However, in the present study the pulvinate *Raoulia* species do not cluster together. The isolated *R. eximia* is quite separated from *R. bryoides* and *R.* "L". A possible relationship with *Haastia* is indicated. Since this split of *R. eximia* from the other species of *Raoulia* subg. *Psychrophyton* was not obvious in previous studies, the flavonoid data should be applied carefully. *R. bryoides* and *R.* "L" are in the flavonoid study isolated either individually or as a pair.

In summary, the present chemotaxonomic study supports Ward's conclusions (1981) that *Raoulia* subg. *Raoulia* and the pulvinate species of *Raoulia* subg. *Psychrophyton* are widely separated and that some species now included in *Raoulia* have closer links to other genera. The main differences from Ward's results are that the flavonoid studies suggest more strongly the exclusion of *Raoulia glabra* from *Raoulia* (and its possible alliance with *Helichrysum depressum*) and that *R. cinerea* has its closest affinities to *R.* "M" and *Gnaphalium traversii* rather than to *Helichrysum filicaule.*

3.5. Conclusion

In conclusion it may be said that the flavonoid patterns conflict in many ways with the current classification of the genera. They mostly support Ward's findings (1981), but show some differences as well. If the flavonoid pattern supports relationships proposed by other characters, this relationship is more likely to be meaningful, but if the flavonoid pattern discovers new associations or contradicts recognised relationships, the flavonoid data have to be carefully judged. In such cases further research is suggested.

The results of this taxonomic survey of the flavonoids of the Gnaphaliinae, although of limited biochemical value, can nevertheless be usefully applied to a revision of the current taxonomy to help clarify the intrageneric and intergeneric uncertainties.

CHAPTER FOUR

COMBINED LEAF ANATOMICAL AND CHEMOTAXONOMIC ANALYSIS

4.1. Introduction

This chapter presents a synthesis of the leaf anatomical and chemotaxonomic studies. All characters used in these two studies are combined in a joint numerical analysis. The differences between the combined analysis and the analyses of each field are discussed. General conclusions for the taxonomy of the Gnaphaliinae are drawn.

4.2. Methods

For the combined numerical analysis the basic data matrix was formed by 48 species (OTUs) of the Gnaphaliinae and 87 characters. All characters from both the anatomical and the chemical studies were used. *Gnaphalium umbricola* was not included in the analysis since material was not available for chemotaxonomic studies.

Similarities between species were calculated using Gower's general coefficient of similarity. The chemical character states were compared using the simple matching coefficient (see chapter 3). The similarity values were clustered by the unweighted pair group method using arithmetic averages (UPGMA). The degree of fit of the phenogram to the similarity matrix from which it was derived was measured using the cophenetic correlation coefficient of Sokal and Rohlf (1962). (Terminology is explained in chapter 2.)

The program used for the numerical analysis was again "Gower", written by Drs. C.M. Frampton, G.A. Findlay and J.M. Ward, Christchurch.

For comparison of the numerical analyses of the leaf anatomical, the chemotaxonomic and the combined data, the phenograms obtained by UPGMA clustering (and in the case of the chemical data, the single matching coefficient) are used.

4.3. Results

Numerical analysis of the combined data set of leaf anatomical and chemotaxonomic characters

In the phenogram formed by using UPGMA (shown in Figure 4.1.), there are three major clusters: a, b and c.

Cluster a includes all *Leucogenes* species. The paired *L.* "Marlborough" and *L.* "Peel" join with *L. leontopodium* at 0.88 and with *L. grandiceps* at 0.80.

Cluster b has three distinct components. The first includes all New Zealand species of *Anaphalis* and the isolated *Helichrysum filicaule*, the second joins the paired species of *Haastia* with the isolated *Helichrysum lanceolatum* at 0.82 and the third unites the paired *Anaphalis triplinervis* and *Gnaphalium involucratum* with *Pseudognaphalium luteoalbum* at the low similarity level of 0.79.

Cluster c, containing all the remaining species, is clearly divided into cluster d and e.

Cluster d joins, at the similarity level of 0.81, the paired Tasmanian species of *Cassinia* (*C. aculeata* and *C. longifolia*), with the cluster formed by the New Zealand species of *Cassinia* (*C. fulvida* and *C. leptophylla*) and the Tasmanian species of *Helichrysum* sect. *Ozothamnus* (*H. backhousii* and *H. obcordatum*).

Cluster e comprises the four distinct clusters f, g, h and i.

Within cluster f, the paired *Ewartia meredithae* and *E. planchonii* join *E. catipes* at 0.91. This cluster is joined by the paired *Gnaphalium mackayi* and *G. nitidulum* at 0.86 and then by the remotely paired *R. glabra* and *R. petriensis* at 0.84. The united *Raoulia bryoides*, *R.* "L", *R. eximia* and *R. hectori* join in at 0.83 and the final entry into cluster f is *Raoulia grandiflora* at 0.81.

	OTU	Phenogram linkage levels
22	He.coralloides	1.0000
27	He.intermedium	0.9655
30	He.parvifolium	0.9304
23	He.depressum	0.9533
25	He.dimorphums	0.7831
36	Pterygopappus	0.7669
43	R.hookeri	0.9512
47	R.tenuicaulis	0.8379
48	Genus "Z"	0.7784
21	He.bellidioides	0.8881
24	He.dimorphumn	0.8717
45	R."M"	0.8209
13	E.sinclairii	0.8711
17	G.traversii	0.8304
38	R.cinerea	0.7950
37	R.bryoides	0.9518
44	R."L"	0.9164
39	R.eximia	0.9099
42	R.hectori	0.8276
40	R.glabra	0.8615
46	R.petriensis	0.8366
15	G.mackayi	0.9185
16	G.nitidulum	0.8551
11	E.meredithae	0.9080
12	E.planchonii	0.8748
10	E.catipes	0.8120
41	R.grandiflora	0.7516
6	C.aculeata	0.9366
9	C.longifolia	0.8196
7	C.fulvida	0.9857
8	C.leptophylla	0.9571
20	He.backhousii	0.8920
29	He.obcordatum	0.7305
5	A.triplinervis	0.8763
14	G.involucratum	0.7944
35	Pseudognaphalium	0.7466
18	Ha.pulvinaris	0.9272
19	Ha.sinclairii	0.8220
28	He.lanceolatum	0.7674
3	A.subrigida	0.9611
4	A.trinervis	0.9440
1	A.keriensis	0.9579
2	A.rupestris	0.7902
26	He.filicaule	0.6992
33	L."Marlborough"	0.9813
34	L."Peel"	0.8807
32	L.leontopodium	0.7971
31	L.grandiceps	0.0000

Cophenetic correlation = 0.713

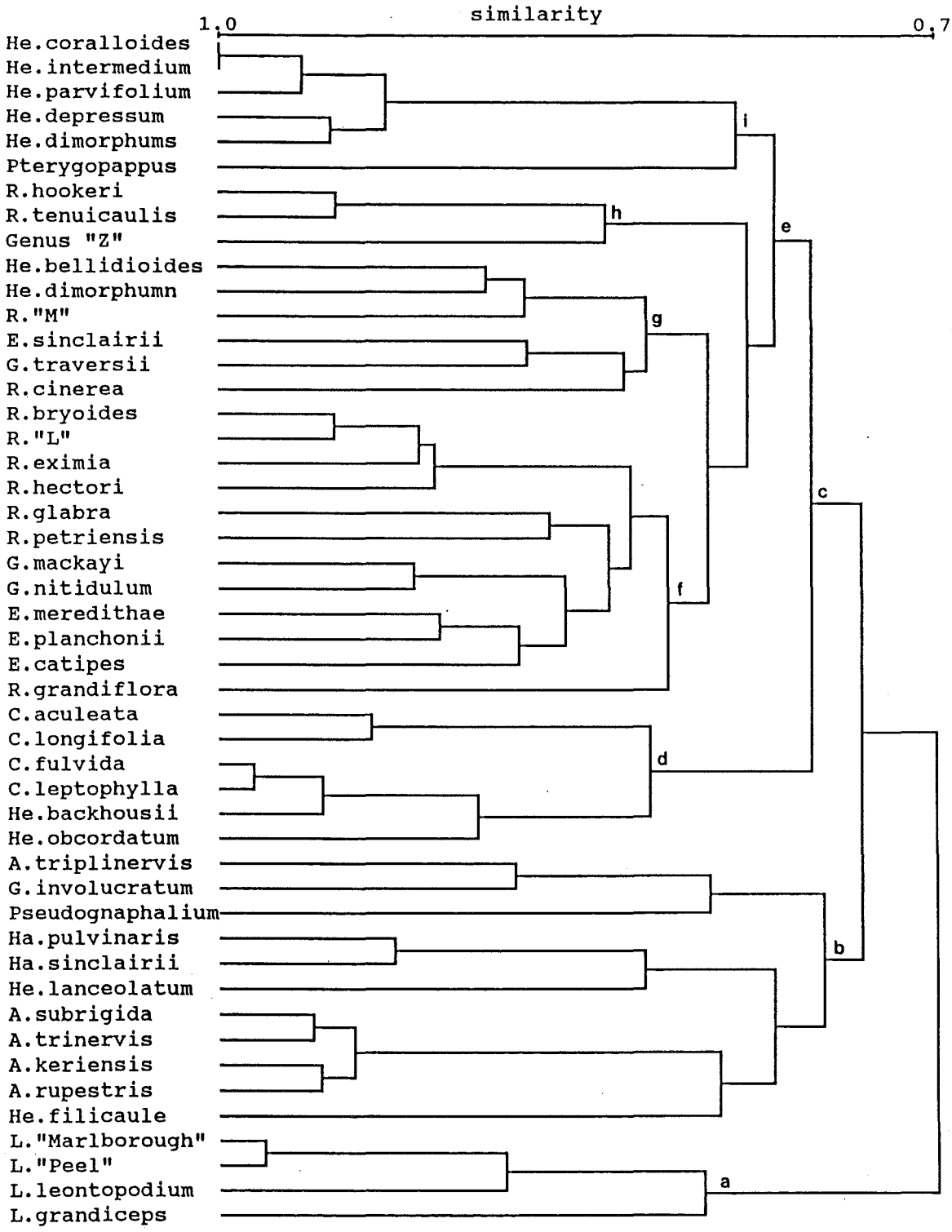


Figure 4.1. UPGMA phenogram from combined data with Gower's coefficient.

Cluster g has six rather isolated species which do however form two loose clusters. In the first, *Ewartia sinclairii* and *Gnaphalium traversii*, paired at 0.87, are joined with *Raoulia cinerea* at the similarity level of 0.83. In the second, *Helichrysum bellidioides* and *Helichrysum dimorphum* (normal leaf), paired at 0.89, are united with *Raoulia* "M" at 0.87. The two clusters are linked at 0.82.

In cluster h, the paired *Raoulia hookeri* and *R. tenuicaulis* are united with Genus "Z" at 0.84.

Cluster i combines *Pterygopappus lawrencii* with the remaining species of *Helichrysum* at a very low similarity level of 0.78. These species of *Helichrysum* form two groups which are united at the very high similarity level of 0.93. One group includes *H. coralloides, H. intermedium* and *H. parvifolium*, the other *H. depressum* and *H. dimorphum* (scale-like leaf).

The cophenetic correlation coefficient has the value of 0.713. The similarity matrix is given in Appendix 6.

In considering the genera, it is apparent that of the 8 represented by more than one species, only *Leucogenes* and *Haastia* form distinct generic clusters. The New Zealand species of *Anaphalis* are closely linked but quite distant from *Anaphalis triplinervis*.

Helichrysum is represented in this study by 10 species in two sections. The species of *Helichrysum* sect. *Xerochlaena* (*H. bellidioides* and *H. filicaule*) are not closely linked. The New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum* and *H. dimorphum* (normal leaf), cluster together. *H. lanceolatum* has no links to any other *Helichrysum* species. The Tasmanian species of *Helichrysum* sect. *Ozothamnus* (*H. backhousii* and *H. obcordatum*) cluster with the New Zealand species of *Cassinia*, while the Tasmanian species of *Cassinia* form a cluster on their own. The Tasmanian species of *Ewartia* are united but clearly separated from the New Zealand species. The species of *Gnaphalium* do not form a coherent group. Only *G. mackayi* and *G. nitidulum* are linked together, while *G. traversii* and *G. involucratum* have affinities to other species. In *Raoulia*, *R. hookeri* and *R. tenuicaulis* have high similarities with one another as do *R. eximia*, *R. bryoides*, *R.* "L" and *R. hectori*. *R. glabra* and *R. petriensis* are

remotely paired, while the remaining species (*R. grandiflora, R. cinerea* and *R.* "M") all have different affinities.

Comparison of the numerical analyses of the leaf anatomical data, the chemotaxonomic data and the combined data

To facilitate comparison, the phenograms of the leaf anatomical data and of the flavonoid data are included again here (Figures 4.2 and 4.3).

There are only few consistent clusters. The species of *Haastia* are paired in all three phenograms. The New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum*, are always joined at high similarity levels. *H. dimorphum* (normal leaf), however, is paired in the leaf anatomy analysis with the Tasmanian *H. obcordatum* and in the combined data analysis with *H. bellidioides*, while in the chemotaxonomic analysis it is identical to its other leaf form. The Tasmanian species of *Cassinia* are paired, while the New Zealand species of *Cassinia* are associated with either both (Figure 4.1) or one of the Tasmanian species of *Helichrysum* sect. *Ozothamnus*. The other Tasmanian species, respectively *H. backhousii* in Figure 4.3 and *H. obcordatum* in Figure 4.2 are also quite similar, but have closer links to other species. The New Zealand species of *Anaphalis* form a consistent cluster. *Helichrysum bellidioides* and *Helichrysum filicaule* are members of this cluster only in the flavonoid analysis. In the other two phenograms, *H. filicaule* has distant links to this cluster (Figure 4.1) or is quite isolated (Figure 4.2) and *H. bellidioides* is either remotely paired with *Raoulia* "M" or clustered with *H. dimorphum* (normal leaf) and *R.* "M".

The species of *Leucogenes* form a distinct cluster in two of the phenograms, but in the third, the flavonoid analysis, *L. grandiceps* has different affinities and *L. leontopodium* is linked only remotely with the remaining two species. *Raoulia tenuicaulis* and *R. hookeri* are always paired. They are joined in Figure 4.3 and 4.1 by Genus "Z", which however has an isolated position in Figure 4.2. *Raoulia bryoides* and *R.* "L" are paired in all three phenograms. *R. eximia* and *R. hectori* join these two species in Figure 4.2 and 4.1., while they each have different affinities in Figure 4.3. The remaining species have changing associations. *Gnaphalium mackayi* and *G. nitidulum* are paired in Figure 4.2 and 4.1, as are *Ewartia meredithae* and *E. planchonii*.

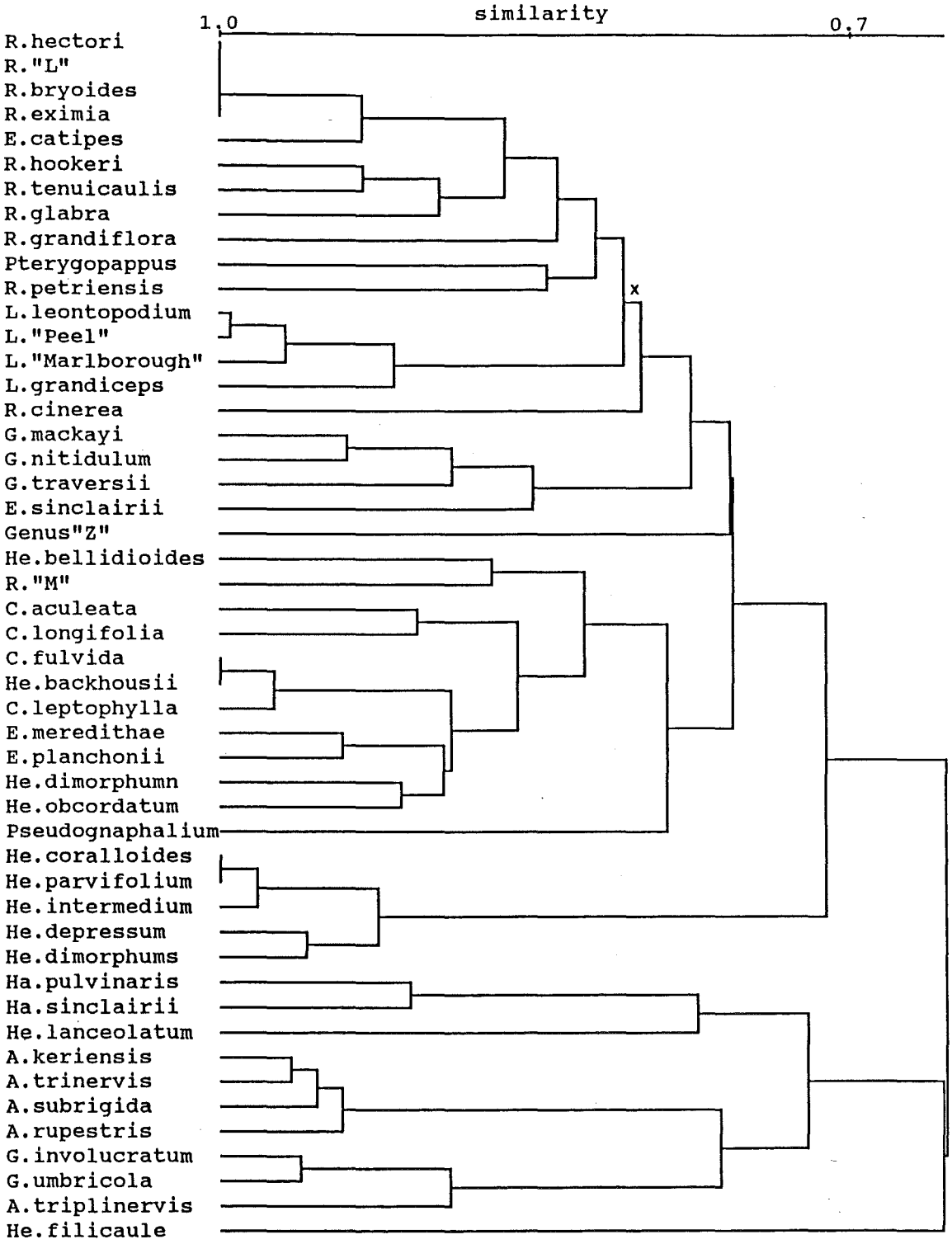


Figure 4.2. UPGMA phenogram from anatomical data with Gower's coefficient.

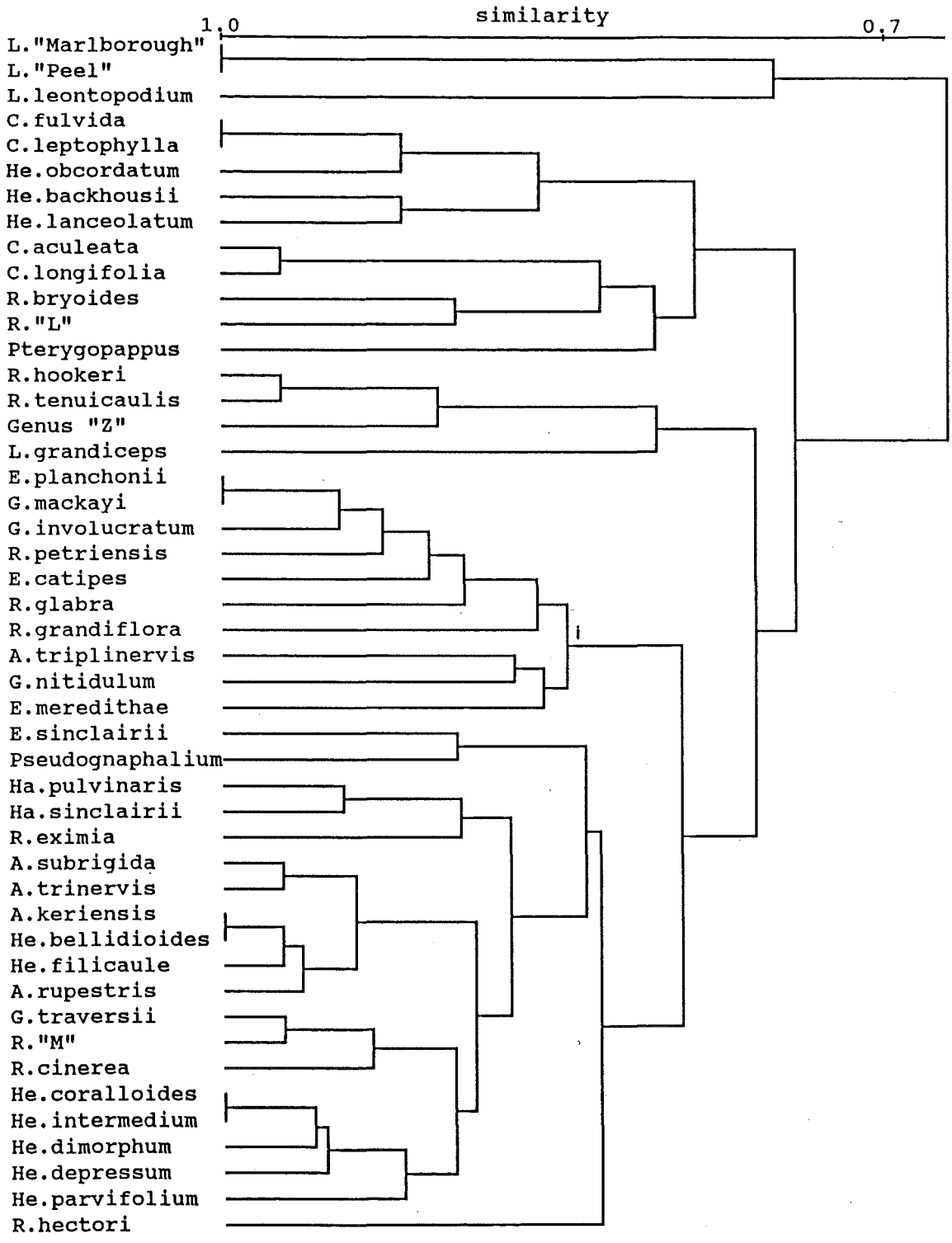


Figure 4.3. UPGMA phenogram from flavonoid data with simple matching coefficient.

Though not as closely related in Figure 4.3, both pairs are members of the ten-species cluster i. *Gnaphalium involucratum* and *Anaphalis triplinervis* similarly are clustered in Figure 4.2 and 4.1 and are members of cluster i in Figure 4.3. *Ewartia catipes* joins the other two Tasmanian *Ewartia* species in Figure 4.1 and is also included in cluster i of Figure 4.3, but clusters with four species of *Raoulia* subg. *Psychrophyton* in Figure 4.2. *Ewartia sinclairii* and *Gnaphalium traversii*, paired in Figure 4.1, are again associated in Figure 4.2 in a cluster with *Gnaphalium mackayi* and *G. nitidulum*, but have quite different affinities in Figure 4.3. *Raoulia glabra*, *R. petriensis* and *R. grandiflora* are all in Figure 4.3 members of cluster i, in Figure 4.1 of cluster f and in Figure 4.2 of cluster x, but they are not very closely associated. *Raoulia cinerea* clusters with the paired *Gnaphalium traversii* and *Raoulia* "M" in Figure 4.3 and with the paired *Ewartia sinclairii* and *Gnaphalium traversii* in Figure 4.1, but is isolated in Figure 4.2. *Pterygopappus lawrencii* and also *Pseudognaphalium luteoalbum* have different and usually remote links in each of the three phenograms.

Considering now the genera, only *Haastia* forms a distinct generic cluster in all three cases. The species of *Leucogenes* are closely related. In the flavonoid study, however, *L. grandiceps* is separated from the other *Leucogenes* species and *L. leontopodium* is only remotely associated. The New Zealand species of *Anaphalis* are always grouped together (although not exclusively in Figure 4.3), but are quite distant from *Anaphalis triplinervis*. *Helichrysum* is represented in this study by two sections, *Helichrysum* sect. *Xerochlaena* and *Helichrysum* sect. *Ozothamnus*. The two species of *Helichrysum* sect. *Xerochlaena* (*H. bellidioides* and *H. filicaule*) are associated in the flavonoid study with the New Zealand species of *Anaphalis*, but are not close to one another in the other phenograms. *H. bellidioides* has links to *Raoulia* "M" and to *Helichrysum dimorphum* (normal leaf), while *H. filicaule* is either remotely associated with the *Anaphalis* group or has no close affinities. The New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum*, cluster together in all three phenograms. *H. lanceolatum* shows no consistent links with other taxa. The normal leaf of *H. dimorphum* has affinities in its leaf anatomy to the Tasmanian *H. obcordatum* and is quite separate from its scale-like leaf, with which it is identical in the flavonoid study. The New Zealand species of *Cassinia* cluster with either one or both of the Tasmanian species of *Helichrysum* sect. *Ozothamnus*. The Tasmanian species of *Cassinia* form a cluster on their own, but associated

with the clusters containing the New Zealand species. The sole New Zealand species of *Ewartia* is clearly separated from the three Tasmanian species of *Ewartia*. Except in the combined analysis, not even the Tasmanian species are very closely associated. In the flavonoid study they are loosely associated (in cluster i), while in the leaf anatomical study, *E. meredithae* and *E. planchonii* are very similar, but *E. catipes* has its closest affinities with *Raoulia* subg. *Raoulia*. *Gnaphalium* also lacks cohesion. In the leaf anatomical study, *G. mackayi, G. nitidulum* and *G. traversii* are associated while *G. involucratum* is quite removed. In the flavonoid study all species except *G. traversii* are loosely associated. The combined analysis joins *G. mackayi* and *G. nitidulum* only. Nor does *Raoulia* form a single group. The species of *Raoulia* subg. *Psychrophyton*, except *R. grandiflora*, are closely associated in the combined analysis and also in the leaf anatomical studies. In the flavonoid studies only *R. bryoides* and *R.* "L" are combined, while *R. eximia* and *R. hectori* have affinities to species of other genera. *R. grandiflora* and *R. petriensis* have changing relationships. In *Raoulia* subg. *Raoulia*, *R. hookeri* and *R. tenuicaulis* are always closely related, while *R. glabra* has changing affinities. *R.* "M" and *R. cinerea* have links either with one another or with species outside *Raoulia*.

4.4. General discussion

Haastia is the only genus in this study which constantly forms a generic cluster. *Haastia* has traditionally been placed in the Astereae, but Merxmüller *et al.* (1977) transferred the type species (*Haastia pulvinaris*) to the Inuleae, commenting that the other two species "seem to represent quite another genus". *H. pulvinaris* was tentatively assigned to the "*Gnaphalium* group", possibly close to *Pterygopappus*. However, none of the evidence presented here reveals any close affinities of the *Haastia* species to *Gnaphalium, Raoulia, Ewartia, Leucogenes* or *Pterygopappus*. It is also proposed in Merxmüller *et al.*'s review that *Haastia pulvinaris* and the two other species of this genus might not be congeneric. As already mentioned, *Haastia pulvinaris* and *H. sinclairii* are always closely linked in the numerical analyses. But, in spite of this high similarity, there is one important character which distinguishes these two species and which suggests that the *Haastia* species might be heterogeneric; this is the presence of secretory ducts in the leaves of *Haastia pulvinaris* (see chapter 2). However, this thesis provides no support for the inclusion of *Haastia* in the *Gnaphalium* group. The other possibility suggested by Merxmüller *et al.*, namely that *H. pulvinaris* might belong in the "*Schoenia* group", should be investigated.

Leucogenes is a small endemic genus with two species recognised by Allan (op. cit.) and two more yet to be described (Molloy, pers. comm.). The species of *Leucogenes* are closely related, although in the flavonoid analysis, *L. grandiceps* is more similar to *Raoulia petriensis* and some other *Raoulia* species than to the other species of *Leucogenes*. A possible reason for the unexpectedly distant links of *Leucogenes leontopodium* with *L.* "Marlborough" and *L.* "Peel" in the flavonoid analysis is given in chapter 3. The analysis of the combined data, however, underlines that *Leucogenes* is a homogeneous group. *L. leontopodium, L.* "Marlborough" and *L.* "Peel" seem to be more closely related to one another than to *L. grandiceps*. Monophyletic origin of *Leucogenes* is also supported by the cladistic analysis of chapter 2.

The New Zealand species of *Anaphalis* are always closely grouped together and are quite distant from the Himalayan *Anaphalis triplinervis*. The New Zealand species of *Anaphalis*, treated by Allan (1961) in *Gnaphalium*, were transferred by Webb (1987) into *Anaphalis*. Webb agreed with Drury (1970) who proposed this transfer based on the subdioecy of these species. *Anaphalis triplinervis*, originating in the Himalayas and grown in New Zealand as a garden plant, was included in this study to investigate the relationship of the New Zealand *Anaphalis* species with a "true" *Anaphalis*. The studies of this thesis definitely support the separation of the New Zealand *Anaphalis* species from *Gnaphalium*. But the New Zealand *Anaphalis* species are not much closer to *Anaphalis triplinervis* than to *Gnaphalium* and *Anaphalis triplinervis* is even closer to the *Gnaphalium-Ewartia* group than to the New Zealand species of *Anaphalis*. Therefore it has to be doubted that the transfer of the anaphalioid group of *Gnaphalium* into *Anaphalis* was a significant improvement for the taxonomy of this group. According to the present study, the New Zealand species of *Anaphalis* do not sit comfortably with any of the other investigated genera but are a group on their own, as was suggested by Bentham (1873b), who placed them in a separate section of *Gnaphalium*, sect. Anaphalioides.

Helichrysum is represented in this study by two sections: *Helichrysum* sect. *Xerochlaena* and *Helichrysum* sect. *Ozothamnus*.

Helichrysum sect. *Xerochlaena* contains two species (*H. bellidioides* and *H. filicaule*). They are associated in the phenograms of the flavonoid data with the New Zealand species of *Anaphalis*, but have different links in the other phenograms. However, *H. bellidioides* and *H. filicaule* are always more similar to one another than to any other species, even if this is not obvious in the phenograms of the leaf anatomy and the combined data set. The highest similarity coefficient of *H. filicaule* is with *H. bellidioides*. But since *H. filicaule* is quite isolated and the similarity coefficients with *H. bellidioides* are relatively low, *H. filicaule* is "forced" to join the cluster with other groups, for example with the New Zealand species of *Anaphalis*, although this link does not reflect the closest relationship. This is a common effect with isolated OTUs in complete linkage clustering (Ward, 1981) and to a lesser extent in the average linkage clustering used here. The results of the studies in this thesis therefore indicate that *H. bellidioides* and *H. filicaule* are indeed closely related. Drury (1971) noted that *H. bellidioides* is probably an

anaphalioid cudweed, but it was not transferred by Webb into *Anaphalis* because it hybridises freely with other species currently treated in New Zealand within *Helichrysum* and more research is required to determine its correct position (Webb, 1987). Ward's phenetic analysis (1981) also reveals close links of *H. bellidioides* with *Anaphalis*. Nothing was noted about *H. filicaule* in Drury's paper. Ward's phenograms (1981) indicate links to *Raoulia cinerea* and not a close relationship of *H. filicaule* with *H. bellidioides*. The results of the present study differ. According to the leaf anatomy and flavonoid data *Helichrysum* sect. *Xerochlaena* is a distinct entity. The relationships of this group remain ambiguous since the species of *Helichrysum* sect. *Xerochlaena* are grouped with *Anaphalis* in the flavonoid study but not in the leaf anatomy study.

Helichrysum sect. *Ozothamnus* is represented in this study by six New Zealand species (*H. coralloides, H. depressum, H. dimorphum, H. intermedium, H. lanceolatum* and *H. parvifolium*) and two Tasmanian species (*H. backhousii* and *H. obcordatum*).

The New Zealand species of *Helichrysum* sect. *Ozothamnus*, except *H. lanceolatum*, are always grouped together. *H. coralloides, H. intermedium* and *H. parvifolium* always have very high similarity indices or are even identical. They have no close affinities to the Tasmanian species of *Helichrysum* sect. *Ozothamnus* nor to any other species of the Gnaphaliinae studied, except *H. depressum* and *H. dimorphum*. The leaf anatomy and the flavonoid study therefore support Ward's decision (pers. comm.) to separate the *Helichrysum* species with imbricate leaves from *Helichrysum*.

The question is now, whether *H. depressum* and *H. dimorphum* should be included in this separated group or not. *H. depressum* is in all three phenograms grouped with *H. coralloides, H. intermedium* and *H. parvifolium*, but it has in the flavonoid study also a high similarity to *Raoulia glabra*, an affinity which was already mentioned in Ward's thesis (1981). Nevertheless, the present study provides more indication for a relationship of *H. depressum* with the New Zealand species of *Helichrysum* sect. *Ozothamnus* than with other species of the Gnaphaliinae.

Helichrysum dimorphum has two leaf morphs, normal leaves and scale-like leaves. The normal leaf has in the leaf anatomy analysis affinities with the Tasmanian *H. obcordatum* and in the combined analysis with *H. bellidioides*, while the scale-like leaf has affinities with the New

Zealand species of *Helichrysum* sect. *Ozothamnus*, (*H. coralloides, H. depressum, H. intermedium* and *H. parvifolium*). In the flavonoid analysis both leaf morphs have an identical spot pattern, which is most similar to that of the New Zealand species of *Helichrysum* sect. *Ozothamnus* excluding *H. lanceolatum*. Botanists have long discussed the origin and position of *H. dimorphum*. Wall in 1919 suggested a hybrid origin with *H. depressum* and *H. filicaule* as the parents, but Allan (1961) notes that progeny tests in the Edinburgh Botanic Gardens and by W.B. Brockie show that it comes "true" from seed. The flavonoid studies do not support the "hybrid hypothesis" (see chapter 3). The anatomy studies neither support nor reject this theory, but suggest that if it is a hybrid, *H. filicaule* is not a very likely parent. The origin and position of this strange species remains obscure, but a very interesting contribution to knowledge of *H. dimorphum* was obtained.

Helichrysum lanceolatum shows no consistent links with any of the other species, nor are any relationships mentioned in the literature. It hybridises apparently with *H. bellidioides*, since *H. purdiei* is supposedly a hybrid between these two species (Allan,1961). Allan notes that similar hybrids have been met with where *H. filicaule* grows in company with *H. lanceolatum*. This thesis could not discover any relationships of *H. lanceolatum*, but underlines its isolated position.

As mentioned above, the Tasmanian species of *Helichrysum* sect. *Ozothamnus* (*H. backhousii* and *H. obcordatum*) are in this study quite separated from the New Zealand species of this section. The Tasmanian species were included in this study not only to investigate whether the New Zealand species of this section are placed correctly within *Helichrysum* sect. *Ozothamnus*, but also to investigate the relationship of the New Zealand *Cassinia* to *Helichrysum*. Hooker (1864) noted that *Ozothamnus* had the characters of *Cassinia*, but without any scales among the florets. He also noted that *Cassinia fulvida* Hook. f., lacking these scales, might be more correctly placed in *Ozothamnus*, and that *Cassinia vauvilliersii* (Homb. et Jacq.) Hook. f. (formerly *Ozothamnus vauvilliersii*) was scarcely distinguishable from a true *Ozothamnus* of Tasmania, *O. cuneifolius* A.C.. Two Tasmanian species of *Cassinia* (*C. aculeata* and *C. longifolia*) were also studied. The results of the present study clearly demonstrate that the New Zealand species of *Cassinia* (*C. fulvida* and *C. leptophylla*) are much closer to the

Tasmanian *Helichrysum* sect. *Ozothamnus* than to the Tasmanian *Cassinia*. This study therefore strongly suggests that the New Zealand species of *Cassinia* would be more appropriately placed in *Helichrysum* than in *Cassinia*.

Ewartia was erected by Beauverd (1910) to contain the subdioecious species of *Raoulia*. The sole New Zealand species, *Ewartia sinclairii*, is however clearly separated in the present study from the three Tasmanian species of this genus (*E. catipes, E. meredithae* and *E. planchonii*). This thus supports Ward's (1981) suggestion that *Ewartia sinclairii* is not congeneric with the Tasmanian species of *Ewartia*. In Ward's thesis (op. cit.), *Ewartia sinclairii* is isolated, with only a remote connection to *Pseudognaphalium luteoalbum*. *Ewartia sinclairii* is also quite isolated in the present study, with links either to *Pseudognaphalium luteoalbum* or to *Gnaphalium traversii*, *G. mackayi* and *G. nitidulum*.

The Tasmanian species of *Ewartia* were included in the present study not only to investigate the position of the New Zealand *Ewartia sinclairii*, but also because of their own uncertain taxonomic position. Ward (1981) suggested that *Ewartia catipes* and *E. planchonii* are closer to one another than to *E. meredithae*. This relationship was also obvious in the flavonoid study, but in the leaf anatomy study *E. planchonii* is closer to *E. meredithae* than to the quite different *E. catipes*. All three species are loosely associated in the combined analysis. These results do not indicate a consistent grouping within *Ewartia*, but show that they are not very closely related. Ward (op. cit.) suggests a relationship between the Australian species of *Ewartia* and the gnaphalioid group of *Gnaphalium*, as well as the relationship with the *Leucogenes-Raoulia grandiflora* & *R. youngii-R. petriensis* alliance. The *Gnaphalium* relationship is also obvious here in the flavonoid study and the combined analysis. But in the leaf anatomy study *E. catipes* is closest to *Raoulia* subg. *Psychrophyton*, while the other two *Ewartia* species have affinities with the Tasmanian species of *Helichrysum* sect. *Ozothamnus* and the New Zealand *Cassinia*. These affinities have to be carefully evaluated since links to this group have not been encountered by studying other characters. Further investigation is needed in this area.

Since *Gnaphalium luteo-album* was transferred into *Pseudognaphalium* (Hilliard and Burtt, 1981) and the anaphalioid group of *Gnaphalium* into *Anaphalis* (Webb, 1987), all

indigenous New Zealand species of *Gnaphalium* belong to section *Euchiton*. However, the four species examined (*G. involucratum, G. mackayi, G. nitidulum* and *G. traversii*) lack cohesion in the present study. *G. mackayi* and *G. nitidulum* seem to be the most closely related species of this group, since they are associated in all three analyses. The other two species are sometimes grouped with the first two *Gnaphalium* species or sometimes show different affinities. This is quite interesting, since *G. mackayi* was included by Allan (1961) in *G. traversii*. The present study underlines Drury's (1972) separation of *G. mackayi* from *G. traversii*. Since the lack of cohesion of *Gnaphalium* sect. *Euchiton* was obvious in the flavonoid and also in the leaf anatomy study, further investigations are required.

Ward (1981), who studied only *G. mackayi* and *G. nitidulum* of *Gnaphalium* sect. *Euchiton*, because of their resemblance to *Raoulia*, found affinities with *Raoulia* subg. *Raoulia* and with the Australian species of *Ewartia*, but not with *Gnaphalium luteo-album* and the anaphalioid group of *Gnaphalium* which were still included in *Gnaphalium* at the time of Ward's studies. In the combined analysis and the flavonoid analysis, *G. mackayi* and *G. nitidulum* are closest to the Tasmanian species of *Ewartia*. In the leaf anatomy study they are closer to *Ewartia sinclairii* than to any other species. When *Gnaphalium traversii* is not grouped with the other *Gnaphalium* species, it has links either to *Raoulia* "M" and *R. cinerea* or to *Ewartia sinclairii* and *Helichrysum dimorphum* (normal leaf). Its position remains unclear, but the present study suggests the species with which it should be compared by analysis of more characters. *Gnaphalium involucratum* is in the flavonoid study quite similar to *G. mackayi*, but in the leaf anatomy study to *Anaphalis triplinervis*. But, as in Ward's thesis, the species of *Gnaphalium* sect. *Euchiton* are never very similar to the New Zealand species of *Anaphalis* or to *Pseudognaphalium luteoalbum*. Therefore the present study supports the separation of these two latter groups from *Gnaphalium*.

Pseudognaphalium luteoalbum appears in Ward's studies (1981) rather distantly linked with *Ewartia sinclairii* and some of her phenograms even suggest a more remote link with *Cassinia*. In the present study *Pseudognaphalium* always has low similarities with other species. It is most similar in the flavonoid study to *Ewartia sinclairii*, in the leaf anatomy analysis to *Cassinia fulvida* and *Helichrysum backhousii* and in the combined analysis to *Helichrysum*

dimorphum (normal leaf), *Ewartia sinclairii* and *Ewartia meredithae*. (Note again that the closest relationships of isolated species, as measured by similarity coefficients, are often not shown in UPGMA phenograms because of the use of similarity averages in this clustering method.) *Pseudognaphalium luteoalbum* has changing affinities in the present study, but since the affinities to *Ewartia sinclairii* and *Cassinia* were also noticed in Ward's thesis, distant links to these species may well be present.

In the present study *Raoulia* is not a single group. Ward (1981) states that the genus as presently constituted contains two very different and clearly demarcated species groups, as well as a number of species of uncertain affinities. According to Ward, the pulvinate species of *Raoulia* subg. *Psychrophyton* form a coherent, uniform group, quite separated from *Raoulia* subg. *Raoulia*, which also forms a coherent if internally variable group, provided that *R. cinerea* and possibly *R.* "M" are removed. In the present study as well, the pulvinate species of *Raoulia* subg. *Psychrophyton* form a group. The only exception is in the flavonoid analysis, where *R. eximia* shows different affinities (discussed in chapter 3). In *Raoulia* subg. *Raoulia*, *R. hookeri* and *R. tenuicaulis* are always closely related, but *R. glabra* has changing affinities, thus supporting Ward's suggestion (1981) that *R. glabra* may be misplaced within subg. *Raoulia*. Since *R.* "M" and *R. cinerea* have links with either one another or species outside *Raoulia*, it is suggested that they should be excluded from subg. *Raoulia* and probably from the genus, as already proposed by Ward (1981). *R.* "M" is in the leaf anatomy study most similar to *Ewartia planchonii*, in the flavonoid study to *R. cinerea* and *Gnaphalium traversii* and in the combined analysis to *E. planchonii* and *G. traversii*, thus reinforcing the affinities with *Gnaphalium* and *Ewartia* shown in Ward's phenograms. *R. cinerea* has no close affinities at all. There are some links to *Ewartia sinclairii*, *R.* "M" and *Gnaphalium traversii*, but not to *Helichrysum filicaule* as in Ward's thesis. Considering the remaining species, *R. hectori* is either grouped with the pulvinate species of subg. *Psychrophyton* or is quite isolated. *R. hectori* seems therefore to be closer to the pulvinate species of subg. *Psychrophyton* than to the species of subg. *Raoulia*. *R. grandiflora* has in the present study no close relationships at all. Within *Raoulia* it is closest to the other species of subg. *Psychrophyton* and to *R. petriensis*, but it has also a high similarity with the Tasmanian species of *Ewartia* and in the leaf anatomy study with *Pterygopappus*.

Therefore its position seems to be closer to subg. *Psychrophyton*, but there are also links to species outside *Raoulia*. However, there are no affinities to *Leucogenes*, as found by Ward (1981). As in Ward (1981), *R. petriensis* has no close affinities. In the combined analysis it is closest to *R. hectori* and *R. glabra*, in the flavonoid study to *R. glabra* and *R. grandiflora* and in the leaf anatomy study to *Pterygopappus*. There are links to the Tasmanian species of *Ewartia* and to *Gnaphalium*. In the cladistic analysis, *R. petriensis* is the sister group to the inverse-dorsiventral species of *Helichrysum* sect. *Ozothamnus*. These conflicting links do nothing to resolve the position of *R. petriensis*.

In conclusion, it can be agreed with Ward that *Raoulia hookeri* and *R. tenuicaulis* of subg. *Raoulia* and the pulvinate species of subg. *Psychrophyton* are widely separated, while the remaining species either form a link between these two subgenera or have links to species outside *Raoulia*.

The Tasmanian monotypic *Pterygopappus lawrencii* was included in this study since Merxmüller *et al.* (1977) included it into their *Gnaphalium* group and suggested a position close to *Haastia pulvinaris*. *Pterygopappus*, however, always has a quite isolated position. It has remote, and different, links in all three phenograms, but never to *Haastia*. Merxmüller *et al.*'s proposal therefore finds no support in the present study.

Genus "Z" is according to Ward (pers. comm.) a new, still undescribed genus. It was mentioned in Allan's *Flora* (1961) under *incertae sedis* and was thought to be close to *Haastia sinclairii* or *Leucogenes grandiceps* or hybrid origin was suggested. In the present study, however, it is not close to either of these species and flavonoid analysis in particular does not support the "hybrid hypothesis" (see chapter 3). Genus "Z" is instead quite isolated, with distant links to the two species of *Raoulia* subg. *Raoulia* (*R. hookeri* and *R. tenuicaulis*). Therefore a close relationship with *Haastia sinclairii* or with *Leucogenes grandiceps* finds no support in the present study.

4.3. General conclusion

Leaf anatomy and flavonoid patterns of the Gnaphaliinae of New Zealand and Tasmania suggest the following relationships, which are not always in agreement with existing generic limits.

The New Zealand species of *Anaphalis* are grouped closely together, but are quite distant from a Himalayan species (*Anaphalis triplinervis*). The relationships of the two New Zealand species of *Helichrysum* sect. *Xerochlaena* (*H. bellidioides* and *H. filicaule*) are unresolved. The New Zealand species of section *Ozothamnus* should be treated separately from *Helichrysum*. The New Zealand species of *Cassinia* are very close to the Tasmanian species of *Helichrysum* sect. *Ozothamnus* and not to the Tasmanian species of *Cassinia*. The New Zealand *Ewartia sinclairii* is not congeneric with the Tasmanian species of *Ewartia*. The exclusion of the anaphalioid species of *Gnaphalium* and of *Gnaphalium luteo-album* from *Gnaphalium* is justified. The species of *Gnaphalium* sect. *Euchiton* are not a coherent group. *Raoulia* should be split into two genera with a few species excluded from both. Genus "Z" has close links to neither *Leucogenes* nor *Haastia*. Its independent generic status seems to be justified. The species of *Leucogenes* are closely related. Neither the species of *Haastia* nor *Pterygopappus lawrencii* reveal particularly close relationships to the *Gnaphalium* group of Merxmüller *et al.* (1977).

> "The present classification of the Gnaphaliinae is unsatisfactory because undue reliance has been placed on single characters, such as floret ratios in the capitulum of *Gnaphalium* and *Helichrysum,* and because the genera are apparently still in an active state of evolution. If the group is to be satisfactorily classified into natural taxa, it will be achieved by abandoning the search for readily or strictly defined genera and approaching the problem in terms of natural aggregations of species." (Ward, 1981)

It would be unwise, of course, to base a future classification of the Gnaphaliinae solely on leaf anatomical and chemotaxonomic studies. As already stated in the introduction, the confusion in the classification of the Gnaphaliinae will best be resolved by pursuing as many and diverse fields of investigation as possible. The leaf anatomical and chemotaxonomic studies generally support Ward's extensive analyses (1981, 1982 and unpublished work) based on

morphological characters. Most species aggregations which are unambiguously supported by morphological characters are also supported by the present study. This correlation shows the taxonomic value of leaf anatomy and chemotaxonomic characters in the Gnaphaliinae. In cases where leaf anatomy and chemistry are not in accord with the earlier morphological work, further investigation is required. Species whose relationships were unresolved by the earlier work were unresolved here as well. However, some very tentative suggestions based on morphology gain support here. Information on species not included in Ward's analyses was obtained and relationships were suggested which should be tested in future by using other characters. The exploratory phylogenetic hypothesis presented here should also be tested by using more and different characters.

In conclusion, it may be said that the leaf anatomical and chemotaxonomic studies in this thesis have provided information of positive value in the classification of the Gnaphaliinae.

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APPENDIX 1

Collecting data for leaf anatomy

Anaphalis keriensis (Cunn.) C. Webb. N.Z.: Canterbury, Arthur's Pass, *Breitwieser & Vogt 827*; Kaweka State Forest, Tutaekuri R., *Breitwieser & Vogt 887.*

Anaphalis rupestris C. Webb. N.Z.: N. Otago, Shag Point, *Ward 88364.*

Anaphalis subrigida (Colenso) C. Webb. N.Z.: Ruahine Ra., Mangoia Stream, *Breitwieser & Vogt 862*; Erua State Forest, Makatote Viaduct, *Breitwieser & Vogt 898.*

Anaphalis trinervis (Forst. f.) F. Muell.. N.Z.: Westland, Jacksons, *Breitwieser & Vogt 544*; Westland, Franz Josef, *Breitwieser & Vogt 597.*

Anaphalis triplinervis (Sims) S.B. Clarke N.Z.: Univ. of Canterbury, glasshouses, *Breitwieser & Vogt 836.*

Cassinia aculeata R. Br.. Australia: Tasmania, South Cape, *Breitwieser & Vogt 673*; Tasmania, Hobart, *Breitwieser & Vogt 756*; Tasmania, Bruny Island, *Breitwieser & Vogt 660.*

Cassinia fulvida Hook. f.. N.Z.: Canterbury, Mt. White Station, *Breitwieser & Vogt 826*; Canterbury, Porter's Pass, *Breitwieser & Vogt 828*; Central Otago, Coronet Peak, *Breitwieser & Vogt 584.*

Cassinia leptophylla (Forst.f.) R.Br.. N.Z.: Wellington, Rimutaka Ra., *Breitwieser & Vogt 845*; Marlborough, Ward Beach, *Breitwieser & Vogt 784.*

Cassinia longifolia R. Br.. Australia: Tasmania, Weldborough, *Breitwieser & Vogt 725.*

Ewartia catipes (DC.) Beauverd. Australia: Tasmania, Ben Lomond, *Breitwieser & Vogt 724.*

Ewartia meredithae (F. Muell.) Beauverd. Australia: Tasmania, Cradle Mtn., *Breitwieser & Vogt 697*; Tasmania, Hartz Peak, *Breitwieser & Vogt 670.*

Ewartia planchonii (Hook. f.) Beauverd. Australia: Tasmania, Mt. Wellington, *Breitwieser & Vogt 738*; Tasmania, Cradle Mtn., *Breitwieser & Vogt 708.*

Ewartia sinclairii (Hook. f.) Cheesem.. N.Z.: Marlborough, Awatere V., *Breitwieser & Vogt 786*; Marlborough, Awatere V., *Breitwieser & Vogt 792.*

Gnaphalium involucratum Forst. f.. N.Z.: Canterbury, Esk R., *Breitwieser & Vogt 824.*

Gnaphalium mackayi (Buchanan) Cockayne. N.Z.: Canterbury, Cass Saddle, *Breitwieser & Vogt 810*; Canterbury, Mt. Alford, *Breitwieser & Vogt 833.*

Gnaphalium nitidulum Hook. f.. N.Z.: N. Canterbury, Island Saddle, *Breitwieser & Vogt 638*; Canterbury, Cass Saddle, *Breitwieser & Vogt 811.*

Gnaphalium traversii Hook. f.. N.Z.: N. Canterbury, Lake Tennyson, *Breitwieser & Vogt 801*; Canterbury, Lake Heron, *Breitwieser & Vogt 779.*

Gnaphalium umbricola J.H. Willis. Australia: Tasmania, Cradle Mtn., *Breitwieser & Vogt 701.*

Haastia pulvinaris Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 905*; N. Canterbury, Mt. Maling, *Breitwieser & Vogt 909*.

Haastia sinclairii Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 906*.

Helichrysum backhousii (Hook. f.) F. Muell. ex Benth. Australia: Tasmania, Mt. Wellington, *Breitwieser & Vogt 743*; Tasmania, Hartz Peak, *Breitwieser & Vogt 669*; Tasmania, Ben Lomond, *Breitwieser & Vogt 723*; Tasmania, Cradle Mtn., *Breitwieser & Vogt 698*.

Helichrysum bellidioides (Forst. f.) Willd.. N.Z.: N. Canterbury, Lewis Pass, *Breitwieser & Vogt 547*; Central Otago, Cardrona, *Breitwieser & Vogt 587*; Canterbury, Cass Saddle, *Breitwieser & Vogt 813*; Nelson, No Man Creek, *Breitwieser & Vogt 631*.

Helichrysum coralloides (Hook. f.) Benth. et Hook. f.. N.Z.: Marlborough, Awatere V., *Breitwieser & Vogt 787*; Marlborough, Awatere V., *Ward 88158*.

Helichrysum depressum (Hook. f.) Benth. et Hook. f.. N.Z.: S. Canterbury, Mt. Potts, *Breitwieser & Vogt 776*; Canterbury, Cass R., *Breitwieser & Vogt 765*; S. Canterbury, Mt. Cook, *Breitwieser & Vogt 619*.

Helichrysum dimorphum Ckn.. N.Z.: Canterbury, Waimakariri R., *Breitwieser & Vogt 820*; Canterbury, Poulter R., *Breitwieser & Vogt 770*.

Helichrysum intermedium Simpson. N.Z.: Marlborough, Wairau Gorge, *Breitwieser & Vogt 803*; Canterbury, Poulter R., *Breitwieser & Vogt 825*; Canterbury, Cass R., *Breitwieser & Vogt 817*.

Helichrysum filicaule Hook. f.. N.Z.: Canterbury, Poulter R., *Breitwieser & Vogt 769*; Nelson, Wairau Gorge, *Breitwieser & Vogt 805*; Canterbury, Banks Peninsula, *Ward 84012*.

Helichrysum lanceolatum (Buchanan) Kirk. N.Z.: Canterbury, Port Hills, *Breitwieser & Vogt 809*; Nelson, Abel Tasman Nat. Pk., *Breitwieser & Vogt 781*.

Helichrysum obcordatum (DC.) F. Muell. ex Benth.. Australia: Tasmania, New Norfolk, *Breitwieser & Vogt 675*; Tasmania, Bicheno, *Breitwieser & Vogt 730*.

Helichrysum parvifolium Yeo. N.Z.: N. Canterbury, Jollie's Pass, *Breitwieser & Vogt 797*; Marlborough, Awatere V., *Breitwieser & Vogt 789*.

Leucogenes grandiceps (Hook. f.) Beauverd. N.Z.: Central Otago, Mt. St. Bathans, *Breitwieser & Vogt 613*; Canterbury, Craigieburn Ra., *Breitwieser & Vogt 760*.

Leucogenes leontopodium (Hook. f.) Beauverd. N.Z.: Tararua Ra., Wellington, *Breitwieser & Vogt 847*.

Leucogenes "Marlborough". N.Z.: Cultivated at DSIR, G 6617.

Leucogenes "Peel". N.Z.: Cultivated at DSIR, G 6664.

Pseudognaphalium luteoalbum (L.) Hilliard et B.L. Burtt. N.Z.: Kaweka State Forest, Donald R., *Breitwieser & Vogt 891*; Westland, Gillespies Beach, *Breitwieser & Vogt 600*.

Pterygopappus lawrencii Hook. f.. Australia: Tasmania, Cradle Mtn., *Breitwieser & Vogt 705*; Tasmania, Mt. Field, *Breitwieser & Vogt 682*.

Raoulia bryoides Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 793*; N. Canterbury, Island Saddle, *Breitwieser & Vogt 800*.

Raoulia cinerea Petrie. N.Z.: Marlborough, Mt. Barefell, *Ward 88265*; N. Canterbury, Balaclava Range, *Breitwieser & Vogt 922*.

Raoulia eximia Hook. f.. N.Z.: Canterbury, Mt. Hutt, *Breitwieser & Vogt 831*; S. Canterbury, Mt. Potts, *Breitwieser & Vogt 772.*

Raoulia glabra Hook. f.. N.Z.: N. Canterbury, Lewis Pass, Breitwieser & Vogt 546; Marlborough, Wairau Gorge, *Breitwieser & Vogt 808*; Canterbury, Cass R., *Breitwieser & Vogt 766.*

Raoulia grandiflora Hook. f.. N.Z.: N. Canterbury, Island Saddle, *Breitwieser & Vogt 799*; Canterbury, Mt. Hutt, *Breitwieser & Vogt 830*; Canterbury, Craigieburn Ra., *Breitwieser & Vogt 737.*

Raoulia hectori Hook. f.. N.Z.: Central Otago, Remarkables, *Breitwieser & Vogt 581*; Central Otago, Old Man Range, *Breitwieser & Vogt 566.*

Raoulia hookeri Allan. N.Z.: Canterbury, Esk R., *Breitwieser & Vogt 821*; Canterbury, Cass R., *Breitwieser & Vogt 768*; Marlborough, Wairau Gorge, *Breitwieser & Vogt 806.*

Raoulia "L". N.Z.: Central Otago, Remarkables, *Breitwieser & Vogt 580.*

Raoulia "M". N.Z.: Canterbury, Mt. Hutt, *Breitwieser & Vogt 829*; S. Canterbury, Mt. Potts, *Breitwieser & Vogt 771.*

Raoulia petriensis Kirk. N.Z.: Central Otago, Mt. St. Bathans, *Breitwieser & Vogt 612.*

Raoulia tenuicaulis Hook. f.. N.Z.: Marlborough, Wairau Gorge, *Breitwieser & Vogt 802*; Canterbury, Esk R., *Breitwieser & Vogt 822*; Canterbury, Cass R., *Breitwieser & Vogt 761*; Westland, Clearwater Creek, *Breitwieser & Vogt 609.*

Genus "Z". N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 795*; Marlborough, Mt. Barefell, *Ward 89097.*

APPENDIX 2

Characters for numerical analysis

1. Cuticle: <thickness>
    1.1. adaxial thicker than abaxial
    1.2. abaxial thicker than adaxial
    1.3. adaxial as thick as abaxial

2. Cuticle: <thickness at margin>
    2.1. thicker at margin
    2.2. not thicker at margin

3. Cuticle: <thickness at midrib>
    3.1. thicker at midrib
    3.2. not thicker at midrib

4. Epidermis: <thickness>
    4.1. adaxial thicker than abaxial
    4.2. abaxial thicker than adaxial
    4.3. adaxial as thick as abaxial

5. Epidermis: <average cell height of adaxial epidermis>

6. Epidermis: <average cell height of abaxial epidermis>

7. Epidermis: <cell shape>
    7.1. all cells round or oval
    7.2. abaxial cells mostly rectangular

8. Epidermis: <variation of adaxial epidermis>
    8.1. cell size regular
    8.2. cell size irregular

9. Epidermis: <variation of abaxial epidermis>
    9.1. cell size regular
    9.2. cell size irregular

10. Epidermis: <cell size in abaxial midrib>
    10.1. thicker than the other abaxial epidermal cells
    10.2. same size as the other abaxial epidermal cells

11. Epidermis: <cell modification in adaxial midrib>
    11.1. 4 narrower cells
    11.2. more than 4 narrower cells

12. Epidermis: <cell modification>
    12.1. epidermis normal
    12.2. occasionally two cells instead of one

13. Epidermis: <cell modification in adaxial midrib>
    13.1. none
    13.2. one big cell in adaxial midrib

14. Stomata: <arrangement>
    14.1. only on adaxial side
    14.2. more on adaxial side
    14.3. equal numbers on both sides
    14.4. more on abaxial side
    14.5. only on abaxial side

15. Stomata: <position>
    15.1. level
    15.2. slightly raised
    15.3. raised
    15.4. extremely raised

16. Stomata: <substomatal chambers>
    16.1.small, i.e., less than half the leaf width
    16.2.medium, i.e., half the leaf width
    16.3.large, i.e., more than half the leaf width

17. Mesophyll: <spongy parenchyma>
    17.1. normal spongy parenchyma present
    17.2. normal spongy parenchyma absent

18. Spongy parenchym cells: <shape>
    18.1 different to palisade cells
    18.2 similar to palisade cells

19. Mesophyll:<large polygonal middle cells>
    19.1. present
    19.2. absent

20. Mesophyll: <arrangement>
    20.1. almost homogeneous
    20.2. not homogeneous

21. Mesophyll: <arrangement>
    21.1. poorly differentiated
    21.2. well differentiated

22. Mesophyll: <*if* well differentiated>
    22.1. oval/round/oval cells distinguishable
    22.2. oval/round cells distinguishable

23. Mesophyll: <palisade parenchyma, arrangement>
    23.1. palisade parenchyma only on the abaxial side
    23.2. palisade parenchyma not only on the abaxial side

24. Mesophyll: <palisade parenchyma, arrangement>
    24.1. palisade parenchyma equally on both sides
    24.2. palisade parenchyma not equally on both sides

25. Mesophyll: <middle cells>
    25.1. medium-sized
    25.2. small
    25.3. absent

26. Mesophyll: <arrangement>
    26.1. small round cells next to abaxial epidermis
    26.2. different cells next to abaxial epidermis

27. Mesophyll: <palisade cells, shape>
    27.1. palisade cells rod-shaped
    27.2. palisade cells oval (palisade-like)

28. Mesophyll: <length/width ratio of the palisade cells next to the epidermis>

29. Mesophyll: <length of the palisade cells next to the epidermis>

30. Mesophyll: <arrangement>
    30.1. dorsiventral
    30.2. equifacial

31. Mesophyll: <palisade/spongy parenchyma ratio>

32. Mesophyll: <palisade arrangement at the margin>
    32.1 palisade cells only at one side
    32.2 palisade cells around the peripheries
    32.3.palisade cells absent

33. Midrib: <protruding, in μm>

34. Midvein: <number of bundle-sheath layers>

35. Midrib: <palisade cells in midrib>
    35.1. absent
    35.2. present

36. Midrib: <abaxial collenchyma>
    36.1. present
    36.2. absent

37. Midvein: <sclerenchyma caps>
    37.1. present
    37.2. absent

38. Midvein: <*if* sclerenchyma caps present>
    38.1. adaxial
    38.2. abaxial
    38.3. adaxial and abaxial

39. Midrib: <shape on the not protruding side)
    39.1. straight
    39.2. not straight

40. Midrib: <thick-walled cells in parenchyma>
    40.1. present
    40.2. absent

41. Midvein: <bundle-sheath>
    41.1. thick-walled
    41.2. not thick-walled

42. Lateral ribs: <protruding>
    42.1. yes
    42.2. no

43. Lateral ribs: <mesophyll>
    43.1. collenchyma cells
    43.2. unspecialised

44. Lateral ribs: <bundle-sheath extension>
    44.1. present
    44.2. absent

45. Lateral ribs: <collenchyma>
    45.1. present
    45.2. absent

46. Leaf: <shape>
    46.1. normal leaf
    46.2. crenulate leaf
    46.3. scale-like leaf
    46.4. needle-like leaf

47. Secretory canals
    47.1. present
    47.2. absent

48. Spaces between epidermis and mesophyll
    48.1. present
    48.2. absent

49. Thick-walled cells in mesophyll
    49.1. present
    49.2. absent

APPENDIX 3

Characters for cladistic analysis

1. Cuticle: <thickness> consistency index 0.400
    1.1. adaxial thicker than abaxial
    1.2. abaxial thicker than adaxial
    1.3. adaxial as thick as abaxial

2. Epidermis: <thickness> consistency index 0.333
    2.1. adaxial thicker than abaxial
    2.2. abaxial thicker than adaxial
    2.3. adaxial as thick as abaxial

3. Epidermis: <cell shape> consistency index 1.000
    3.1. all cells round or oval
    3.2. abaxial cells mostly rectangular

4. Stomata: <arrangement> consistency index 0.400
    4.1. only on adaxial side
    4.2. more on adaxial side
    4.3. equal number on both sides
    4.4. more on abaxial side
    4.5. only on abaxial side

5. Stomata: <position> consistency index 0.214
    5.1. level
    5.2. slightly raised
    5.3. raised
    5.4. extremely raised

6. Stomata: <substomatal chambers> consistency index 0.167
    6.1.small, i.e., less than half the leaf width
    6.2.medium, i.e., half the leaf width
    6.3.large, i.e., more than half the leafwidth

7. Mesophyll: <spongy parenchyma> consistency index 0.500
    7.1. normal spongy parenchyma present
    7.2. normal spongy parenchyma absent

8. Mesophyll: <large polygonal middle cells> consistency index 1.000
    8.1. present
    8.2. absent

9. Mesophyll: <arrangement> consistency index 0.500
    9.1. poorly differentiated
    9.2. well differentiated

10. Mesophyll: <*if* poorly differentiated> consistency index 1.000
    10.1. mesophyll with oval/round/oval cells
    10.2. mesophyll with oval/round cells

11. Mesophyll: <palisade parenchyma> consistency index 1.000
    11.1. palisade parenchyma only on the abaxial side
    11.2. palisade parenchyma not only on the abaxial side

12. Mesophyll: <round middle cells> consistency index 0.500
    12.1. present
    12.2. absent

13. Mesophyll: <arrangement> consistency index 0.500
    13.1. small round cells next to abaxial epidermis
    13.2. different cells next to abaxial epidermis

14. Mesophyll: <palisade cells, shape> consistency index 0.143
    14.1. palisade cells rod-shaped
    14.2. palisade cells oval (palisade-like)

15. Mesophyll: <arrangement> consistency index 0.500
    15.1. dorsiventral
    15.2. equifacial

16. Midrib: <protrudes> consistency index 0.250
    16.1. protrudes not
    16.2. protrudes

17. Midrib: <adaxial collenchyma> consistency index 0.500
    17.1. present
    17.2. absent

18. Midrib: <abaxial collenchyma> consistency index 0.333
    18.1. present
    18.2. absent

19. Midvein: <sclerenchyma caps> consistency index 0.250
    19.1. present
    19.2. absent

20. Midvein: <*if* sclerenchyma caps present> consistency index 1.000
    20.1. adaxial
    20.2. abaxial
    20.3. adaxial and abaxial

21. Midrib: <palisade parenchyma in midrib>
consistency index 0.500
21.1. present
21.2. absent

22. Midrib: <shape adaxial> consistency index 0.500
22.1. straight
22.2. not straight

23. Lateral ribs: <collenchyma> consistency index 0.333
23.1. present
23.2. absent

24. Lateral ribs: <palisade cells> consistency index 0.500
24.1. present
24.2. absent

25. Leaf: <shape> consistency index 0.750
25.1. normal leaf
25.2. crenulate leaf
25.3. scale-like leaf
25.4. needle-like leaf

APPENDIX 4

Collecting data for chemotaxonomy

Anaphalis keriensis (Cunn.) C. Webb. N.Z.: Canterbury, Arthur's Pass, *Breitwieser & Vogt 827.*

Anaphalis rupestris C. Webb. N.Z.: N. Otago, Shag Point, *Ward 88364.*

Anaphalis subrigida (Colenso) C. Webb. N.Z.: Ruahine Ra., Oroua R., *Breitwieser & Vogt 861*; Ruahine Ra., Mangoira Stream, *Breitwieser & Vogt 862.*

Anaphalis trinervis (Forst. f.) F. Muell.. N.Z.: Westland, Jacksons, *Breitwieser & Vogt 544*; Westland, Lake Moeraki, *Breitwieser & Vogt 653.*

Anaphalis triplinervis (Sims) C.B. Clarke N.Z.: Univ. of Canterbury, glasshouses, *Breitwieser & Vogt 836.*

Cassinia aculeata R. Br.. Australia: Tasmania, Cradle Mtn., *Breitwieser & Vogt 710.*

Cassinia fulvida Hook. f.. N.Z.: Canterbury, Cass, *Breitwieser & Vogt 828*; Central Otago, Coronet Peak, *Breitwieser & Vogt 584.*

Cassinia leptophylla (Forst.f.) R.Br.. N.Z.: Canterbury, Port Hills, *Breitwieser & Vogt 834.*

Cassinia longifolia R. Br.. Australia: Tasmania, Weldborough, *Breitwieser & Vogt 725.*

Ewartia catipes (DC.) Beauverd. Australia: Tasmania, Ben Lomond, *Breitwieser & Vogt 724.*

Ewartia meredithae (F. Muell.) Beauverd. Australia: Tasmania, Hartz Peak, *Breitwieser & Vogt 670.*

Ewartia planchonii (Hook. f.) Beauverd. Australia: Tasmania, Mt. Wellington, *Breitwieser & Vogt 738.*

Ewartia sinclairii (Hook. f.) Cheesem.. N.Z.: Marlborough, Awatere V., *Breitwieser & Vogt 786.*

Gnaphalium involucratum Forst. f.. N.Z.: Canterbury, Esk R., *Breitwieser & Vogt 824.*

Gnaphalium mackayi (Buchanan) Cockayne. N.Z.: Central Otago, Remarkables, *Breitwieser & Vogt 572.*

Gnaphalium nitidulum Hook. f.. N.Z.: N. Canterbury, Island Saddle, *Breitwieser & Vogt 638.*

Gnaphalium traversii Hook. f.. N.Z.: Central Otago, Cardrona, *Breitwieser & Vogt 589.*

Haastia pulvinaris Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 905*; N. Canterbury, Mt. Maling, *Breitwieser & Vogt 909.*

Haastia sinclairii Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 906.*

Helichrysum backhousii (Hook. f.) F. Muell. ex Benth. Australia: Tasmania, Mt. Field, *Breitwieser & Vogt 688*; Tasmania, Cradle Mtn., *Breitwieser & Vogt 698.*

Helichrysum bellidioides (Forst. f.) Willd.. N.Z.: N. Canterbury, Lewis Pass, *Breitwieser & Vogt 547*; N. Canterbury, Lewis Pass, *Breitwieser & Vogt 548*; Central Otago, Cardrona, *Breitwieser & Vogt 587*; Central Otago, Remarkables, *Breitwieser & Vogt 577.*

Helichrysum coralloides (Hook. f.) Benth. et Hook. f.. N.Z.: Marlborough, Awatere V., *Breitwieser & Vogt 787.*

Helichrysum depressum (Hook. f.) Benth. et Hook. f.. N.Z.: N. Canterbury, Lake Tennyson, *Breitwieser & Vogt 637*; S. Canterbury, Mt. Cook, *Breitwieser & Vogt 619.*

Helichrysum dimorphum Ckn.. N.Z.: Canterbury, Poulter R., *Breitwieser & Vogt 770.*

Helichrysum intermedium Simpson. N.Z.: Canterbury, Cass R., *Breitwieser & Vogt 542.*

Helichrysum filicaule Hook. f.. N.Z.: Canterbury, Poulter R., *Breitwieser & Vogt 769*; Kaweka State Forest, Lawrence Hut, *Breitwieser & Vogt 890.*

Helichrysum lanceolatum (Buchanan) Kirk. N.Z.: Nelson, Abel Tasman Nat. Pk., *Breitwieser & Vogt 781.*

Helichrysum obcordatum (DC.) F. Muell. ex Benth.. Australia: Tasmania, Bicheno, *Breitwieser & Vogt 730.*

Helichrysum parvifolium Yeo. N.Z.: Marlborough, Awatere V., *Breitwieser & Vogt 789.*

Leucogenes grandiceps (Hook. f.) Beauverd. N.Z.: Central Otago, Mt. St. Bathans, *Breitwieser & Vogt 613.*

Leucogenes leontopodium (Hook. f.) Beauverd. N.Z.: Tararua Ra., Wellington, *Breitwieser & Vogt 847.*

Leucogenes "Marlborough". N.Z.: Cultivated at DSIR, G16617.

Leucogenes "Peel". N.Z.: Cultivated at DSIR, G16664.

Pseudognaphalium luteoalbum (L.) Hilliard et B.L. Burtt. N.Z.: Westland, Clearwater Creek, *Breitwieser & Vogt 611*; Westland, Gillespies Beach, *Breitwieser & Vogt 600.*

Pterygopappus lawrencii Hook. f.. Australia: Tasmania, Mt. Field, *Breitwieser & Vogt 677.*

Raoulia bryoides Hook. f.. N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 793.*

Raoulia cinerea Petrie. N.Z.: Marlborough, Mt. Barefell, *Ward 88265*; N. Canterbury, Balaclava Range, *Breitwieser & Vogt 922.*

Raoulia eximia Hook. f.. N.Z.: S. Canterbury, Mt. Potts, *Breitwieser & Vogt 772.*

Raoulia glabra Hook. f.. N.Z.: N. Canterbury, Lewis Pass, *Breitwieser & Vogt 546*; Kaweka State Forest, Tutaekuri R., *Breitwieser & Vogt 883.*

Raoulia grandiflora Hook. f.. N.Z.: Central Otago, Coronet Peak, *Breitwieser & Vogt 586*; Central Otago, Remarkables, *Breitwieser & Vogt 571.*

Raoulia hectori Hook. f.. N.Z.: Central Otago, Mt. St. Bathans, *Breitwieser & Vogt 614.*

Raoulia hookeri Allan. N.Z.: Westland, Clearwater Creek, *Breitwieser & Vogt 610*; S. Canterbury, Mt. Cook, *Breitwieser & Vogt 629*; Westland, Mahitahi R., *Breitwieser & Vogt 654.*

Raoulia "L". N.Z.: Central Otago, Remarkables, *Breitwieser & Vogt 580.*

Raoulia "M". N.Z.: S. Canterbury, Mt. Potts, *Breitwieser & Vogt 771.*

Raoulia petriensis Kirk. N.Z.: Central Otago, Mt. St. Bathans, *Breitwieser & Vogt 612.*

Raoulia tenuicaulis Hook. f.. N.Z.: S. Canterbury, Mt. Cook, *Breitwieser & Vogt 592*; Westland, Gillespies Beach, *Breitwieser & Vogt 607*; Westland, Clearwater Creek, *Breitwieser & Vogt 609*.

Genus "Z". N.Z.: Marlborough, Mt. Barefell, *Breitwieser & Vogt 795*; Marlborough, Mt. Barefell, *Breitwieser & Vogt 907*.

APPENDIX 5

High-Performance Liquid Chromatography (HPLC) Analysis

0.5 g of powdered leaves were homogenised for one minute in 20 ml 75% acetone using an Ultra-Turax homogeniser. The leaves were extracted three more times in this manner. The extractant was filtered through two layers of Miracloth and then reduced *in vacuo* to 20% of its original volume. After centrifuging for 5 min at 1000 G, the extract was filtered through a Whatman GF/C glass fibre filter, adjusted to pH 7 with a 0.1M Na_2HPO_4 buffer and applied to a 10 mm x 15 mm column containing 250 mg of C-18 silica which had been previously equilibrated with the buffer. The column was washed further with 2.5 ml of buffer and then eluted with 2 ml of 80% methanol. The elutant was inspected by TLC on cellulose developed in BAW and 15% aq. acetic acid. The 80% methanol eluate was diluted to 5 ml in a volumetric flask. Samples were filtered through a 0.4 μm PTFE filter prior to HPLC analysis. 70 μl samples were analysed using a Varian 5000 HPLC fitted with a Brownlee 10 cm 5 μm C-18 reverse phase column + 1.5 cm 5 μm C-18 guard column and a UV detector coupled to a Spectra-Physics computing integrator. The solvents used were A, acetic acid / water (2.5:97.5); B, acetonitril / water / acetic acid (490:490:25). A linear gradient was applied, starting with 100% solvent A and finishing with 100% solvent B over a 15 min. period at a flow rate of 2 ml/min. Separations were performed at 30°C and detection was carried out at 280 and 350 nm. Retention times and peak areas were calculated.

APPENDIX 6

Similarity matrices

1. Similarity matrix from anatomical data with Gower's coefficient

2. Similarity matrix from flavonoid data with simple matching coefficient

3. Similarity matrix from flavonoid data with Jaccard's coefficient

4. Similarity matrix from combined data with Gower's coefficient
   (flavonoid data with simple matching coefficient)

	OTU no. ->	1	2	3	4	5	6	7	8	9	10	11	12
1	A.keriensis												
2	A.rupestris	0.9452											
3	A.subrigida	0.9582	0.9350										
4	A.trinervis	0.9668	0.9474	0.9510									
5	A.triplinervis	0.7753	0.7734	0.7805	0.7681								
6	C.aculeata	0.6794	0.6728	0.6730	0.6771	0.6319							
7	C.fulvida	0.6928	0.6848	0.6765	0.6809	0.7397	0.8377						
8	C.leptophylla	0.6885	0.6819	0.6808	0.6766	0.7426	0.8632	0.9745					
9	C.longifolia	0.6664	0.6598	0.6601	0.6642	0.7016	0.9066	0.8672	0.8715				
10	E.catipes	0.6265	0.6133	0.6045	0.6241	0.6241	0.7671	0.8311	0.8266	0.7987			
11	E.meredithae	0.7382	0.7535	0.7316	0.7318	0.7154	0.8913	0.9046	0.9091	0.8987	0.8495		
12	E.planchonii	0.7069	0.6943	0.6859	0.7046	0.6829	0.8337	0.8809	0.8766	0.8639	0.8420	0.9421	
13	E.sinclairii	0.6385	0.6316	0.6361	0.6261	0.7021	0.7499	0.7816	0.7816	0.8474	0.7442	0.7693	0.7431
14	G.involucratum	0.7467	0.7401	0.7432	0.7445	0.8827	0.7009	0.7963	0.7792	0.7706	0.6721	0.7558	0.7288
15	G.mackayi	0.6187	0.6059	0.6073	0.6062	0.6382	0.7399	0.7761	0.7716	0.7707	0.7971	0.7801	0.8256
16	G.nitidulum	0.6282	0.6148	0.6058	0.6258	0.6381	0.7708	0.7929	0.7883	0.8263	0.8070	0.8089	0.8560
17	G.traversii	0.6810	0.6741	0.6654	0.7008	0.7051	0.7158	0.7849	0.7819	0.7690	0.7921	0.7926	0.7844
18	G.umbricola	0.7736	0.7668	0.7627	0.7713	0.9000	0.6921	0.8180	0.7977	0.7645	0.6990	0.7676	0.7558
19	Ha.pulvinaris	0.6814	0.6740	0.6896	0.6680	0.7707	0.7463	0.7301	0.7301	0.7543	0.6671	0.7437	0.7651
20	Ha.sinclairii	0.7690	0.7616	0.7556	0.7557	0.8237	0.7864	0.7701	0.7701	0.7943	0.6966	0.7838	0.7814
21	He.backhousii	0.6928	0.6848	0.6765	0.6809	0.7397	0.8377	1.0000	0.9745	0.8672	0.8311	0.9046	0.8809
22	He.bellidioides	0.7719	0.7434	0.7683	0.7913	0.7496	0.7730	0.8319	0.8362	0.8032	0.8262	0.8560	0.8328
23	He.coralloides	0.5676	0.5577	0.5651	0.5551	0.5489	0.7636	0.7403	0.7403	0.7723	0.7530	0.7608	0.7498
24	He.depressum	0.5625	0.5557	0.5659	0.5503	0.5915	0.7542	0.7554	0.7380	0.7482	0.7370	0.7587	0.7398
25	He.dimorphums	0.5815	0.5749	0.5876	0.5695	0.5887	0.7408	0.7561	0.7390	0.7491	0.7534	0.7742	0.7553
26	He.dimorphumn	0.7184	0.7115	0.7147	0.7161	0.7380	0.8272	0.8848	0.8671	0.8568	0.8154	0.9076	0.8909
27	He.filicaule	0.6493	0.6359	0.6269	0.6469	0.6342	0.6546	0.6561	0.6514	0.6391	0.6762	0.6711	0.7131
28	He.intermedium	0.5640	0.5531	0.5545	0.5521	0.5419	0.7460	0.7499	0.7244	0.7543	0.7347	0.7426	0.7375
29	He.lanceolatum	0.6990	0.6639	0.6553	0.6744	0.6870	0.6881	0.7043	0.6998	0.7189	0.6885	0.6942	0.7319
30	He.obcordatum	0.7197	0.7130	0.7161	0.7174	0.7500	0.8392	0.8968	0.9011	0.8687	0.8247	0.9034	0.8748
31	He.parvifolium	0.5808	0.5503	0.5875	0.5677	0.5675	0.7239	0.6941	0.6988	0.7562	0.6948	0.7191	0.6998
32	L.grandiceps	0.6128	0.6059	0.6047	0.6003	0.5677	0.7283	0.7507	0.7551	0.7814	0.8157	0.7678	0.7395
33	L.leontopodium	0.5598	0.5470	0.5485	0.5474	0.5130	0.6571	0.6933	0.6888	0.7102	0.7671	0.6943	0.7323
34	L."Marlborough"	0.5528	0.5399	0.5414	0.5403	0.5060	0.6500	0.6863	0.6818	0.6809	0.7614	0.6870	0.7251
35	L."Peel"	0.5654	0.5525	0.5540	0.5529	0.5186	0.6626	0.6988	0.6944	0.7157	0.7728	0.7001	0.7380
36	Pseudognaphalium	0.6986	0.6918	0.7075	0.6864	0.7093	0.7945	0.8410	0.8207	0.8223	0.7191	0.8093	0.7750
37	Pterygopappus	0.5707	0.5598	0.5680	0.5570	0.5857	0.6910	0.7684	0.7684	0.7249	0.8531	0.7839	0.7901
38	R.bryoides	0.5730	0.5602	0.5516	0.5707	0.5606	0.7569	0.7732	0.7687	0.7878	0.9264	0.7884	0.8228
39	R.cinerea	0.6481	0.6412	0.6441	0.6356	0.6574	0.7347	0.7680	0.7665	0.8101	0.7786	0.7734	0.7983
40	R.eximia	0.5730	0.5602	0.5516	0.5707	0.5606	0.7569	0.7732	0.7687	0.7878	0.9264	0.7884	0.8228
41	R.glabra	0.6207	0.6138	0.6153	0.6082	0.6243	0.7510	0.7808	0.7779	0.7819	0.8465	0.7916	0.8157
42	R.grandiflora	0.5591	0.5462	0.5376	0.5466	0.5466	0.6613	0.7370	0.7325	0.7144	0.8387	0.7442	0.7770
43	R.hectori	0.5764	0.5661	0.5633	0.5740	0.5696	0.7766	0.7882	0.7882	0.8090	0.9292	0.8106	0.8390
44	R.hookeri	0.5347	0.5215	0.5127	0.5219	0.4992	0.7301	0.7166	0.7121	0.7162	0.7994	0.7295	0.7571
45	R."L"	0.5861	0.5729	0.5641	0.5836	0.5733	0.7741	0.7907	0.7862	0.8057	0.9475	0.8072	0.8419
46	R."M"	0.6974	0.6908	0.6840	0.6952	0.6782	0.7692	0.8079	0.8065	0.7988	0.8060	0.8308	0.8521
47	R.petriensis	0.5722	0.5597	0.5512	0.5600	0.5482	0.6988	0.7245	0.7202	0.7290	0.8217	0.7537	0.7902
48	R.tenuicaulis	0.5894	0.5766	0.5680	0.5770	0.5770	0.7583	0.7896	0.7851	0.7892	0.8721	0.7992	0.8308
49	Genus"Z"	0.5484	0.5356	0.5472	0.5360	0.6023	0.7142	0.7504	0.7460	0.8118	0.7802	0.7480	0.7317

	OTU no. ->	13	14	15	16	17	18	19	20	21	22	23	24
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum	0.7288											
15	G.mackayi	0.8567	0.6798										
16	G.nitidulum	0.8434	0.7027	0.9396									
17	G.traversii	0.8536	0.7669	0.8882	0.8918								
18	G.umbricola	0.7500	0.9623	0.6903	0.7143	0.7749							
19	Ha.pulvinaris	0.7622	0.6982	0.7664	0.7724	0.6617	0.7157						
20	Ha.sinclairii	0.7929	0.7859	0.7658	0.7844	0.7475	0.8033	0.9102					
21	He.backhousii	0.7816	0.7963	0.7761	0.7929	0.7849	0.8180	0.7301	0.7701				
22	He.bellidioides	0.6964	0.8172	0.7276	0.7534	0.8119	0.8225	0.6713	0.7611	0.8319			
23	He.coralloides	0.6288	0.6060	0.6492	0.6664	0.6340	0.5991	0.6317	0.6624	0.7403	0.7382		
24	He.depressum	0.6267	0.6206	0.6596	0.6777	0.6508	0.6408	0.6537	0.6592	0.7554	0.7344	0.9490	
25	He.dimorphums	0.6303	0.6426	0.6773	0.6958	0.6687	0.6585	0.6490	0.6782	0.7561	0.7544	0.9255	0.9592
26	He.dimorphumn	0.7254	0.8240	0.7728	0.8014	0.8360	0.8165	0.7025	0.7923	0.8848	0.8823	0.7616	0.8005
27	He.filicaule	0.5459	0.6776	0.6566	0.6982	0.6612	0.6714	0.6834	0.6942	0.6561	0.7353	0.6605	0.6107
28	He.intermedium	0.6194	0.6100	0.6388	0.6555	0.6258	0.6121	0.6215	0.6507	0.7499	0.7191	1.0000	0.9447
29	He.lanceolatum	0.7643	0.6895	0.7799	0.7883	0.7191	0.7098	0.7404	0.8058	0.7043	0.6912	0.5854	0.5759
30	He.obcordatum	0.7672	0.7893	0.7597	0.7863	0.8241	0.7947	0.7397	0.8274	0.8968	0.8837	0.7587	0.7495
31	He.parvifolium	0.6262	0.6094	0.5906	0.6065	0.5812	0.5958	0.6385	0.6700	0.6941	0.7225	1.0000	0.9172
32	L.grandiceps	0.8256	0.6060	0.8080	0.8150	0.8279	0.6279	0.6336	0.6819	0.7507	0.7655	0.7345	0.7322
33	L.leontopodium	0.7739	0.5546	0.8304	0.8211	0.7829	0.5631	0.6254	0.6373	0.6933	0.7128	0.7128	0.6924
34	L."Marlborough"	0.7446	0.5475	0.8233	0.7963	0.7530	0.5559	0.6237	0.6294	0.6863	0.7056	0.7185	0.6966
35	L."Peel"	0.7795	0.5601	0.8359	0.8211	0.7886	0.5688	0.6254	0.6436	0.6988	0.7185	0.7072	0.6867
36	Pseudognaphalium	0.7616	0.7789	0.7552	0.7606	0.7100	0.7723	0.7592	0.7659	0.8410	0.7135	0.6951	0.6923
37	Pterygopappus	0.6886	0.6240	0.7372	0.7609	0.7393	0.6418	0.6397	0.6552	0.7684	0.7834	0.7442	0.7545
38	R.bryoides	0.7326	0.6075	0.8127	0.8247	0.7234	0.6324	0.6976	0.7022	0.7732	0.7670	0.7322	0.7170
39	R.cinerea	0.7849	0.6989	0.8033	0.8176	0.7471	0.7086	0.7354	0.7223	0.7680	0.7425	0.6575	0.6539
40	R.eximia	0.7326	0.6075	0.8127	0.8247	0.7234	0.6324	0.6976	0.7022	0.7732	0.7670	0.7322	0.7170
41	R.glabra	0.7296	0.6658	0.8409	0.8330	0.7830	0.6769	0.7193	0.7624	0.7808	0.8266	0.7378	0.7317
42	R.grandiflora	0.6742	0.5983	0.7306	0.7848	0.7367	0.6078	0.6194	0.6365	0.7370	0.7575	0.7386	0.7204
43	R.hectori	0.7480	0.6272	0.8071	0.8430	0.7372	0.6443	0.7133	0.7181	0.7882	0.7900	0.7322	0.7353
44	R.hookeri	0.6751	0.5520	0.7556	0.7765	0.6693	0.5607	0.7411	0.6956	0.7166	0.7139	0.6666	0.6527
45	R."L"	0.7492	0.6213	0.8312	0.8444	0.7402	0.6471	0.7155	0.7202	0.7907	0.7848	0.7432	0.7170
46	R."M"	0.6951	0.7231	0.7365	0.7672	0.7535	0.7459	0.6641	0.7279	0.8079	0.8712	0.7293	0.7243
47	R.petriensis	0.6705	0.5842	0.7689	0.8014	0.7128	0.6080	0.6414	0.6577	0.7245	0.7295	0.8282	0.8120
48	R.tenuicaulis	0.7490	0.6286	0.8277	0.8515	0.7450	0.6389	0.7161	0.7206	0.7896	0.7886	0.7209	0.7060
49	Genus"Z"	0.7644	0.6438	0.7382	0.7669	0.7264	0.6484	0.6856	0.6811	0.7504	0.7466	0.6668	0.6593

	OTU no. ->	25	26	27	28	29	30	31	32	33	34	35	36
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	G.umbricola												
19	Ha.pulvinaris												
20	Ha.sinclairii												
21	He.backhousii												
22	He.bellidioides												
23	He.coralloides												
24	He.depressum												
25	He.dimorphums												
26	He.dimorphumn	0.8418											
27	He.filicaule	0.6071	0.6919										
28	He.intermedium	0.9201	0.7633	0.6448									
29	He.lanceolatum	0.5806	0.7074	0.6448	0.5771								
30	He.obcordatum	0.7546	0.9143	0.6947	0.7342	0.7399							
31	He.parvifolium	0.8957	0.7223	0.6059	0.9652	0.5961	0.7219						
32	L.grandiceps	0.7482	0.7430	0.5738	0.7181	0.6673	0.7654	0.6906					
33	L.leontopodium	0.6871	0.6891	0.6097	0.6978	0.6610	0.6991	0.6563	0.9288				
34	L."Marlborough"	0.6912	0.6818	0.6054	0.7018	0.6537	0.6921	0.6607	0.8995	0.9714			
35	L."Peel"	0.6816	0.6949	0.6157	0.6924	0.6668	0.7047	0.6504	0.9232	0.9946	0.9659		
36	Pseudognaphalium	0.7089	0.7891	0.6035	0.7040	0.6677	0.7631	0.6570	0.7363	0.6819	0.6747	0.6876	
37	Pterygopappus	0.7741	0.7708	0.6277	0.7442	0.6171	0.7886	0.7166	0.8176	0.7879	0.7940	0.7818	0.6611
38	R.bryoides	0.7112	0.7445	0.6712	0.7214	0.7113	0.7669	0.6821	0.8025	0.8154	0.8085	0.8208	0.7078
39	R.cinerea	0.6717	0.7449	0.5661	0.6475	0.6570	0.7546	0.6310	0.8022	0.8112	0.8042	0.8057	0.7613
40	R.eximia	0.7112	0.7445	0.6712	0.7214	0.7113	0.7669	0.6821	0.8025	0.8154	0.8085	0.8208	0.7078
41	R.glabra	0.7477	0.8055	0.6800	0.7250	0.7221	0.8104	0.6901	0.8116	0.8172	0.8101	0.8227	0.7275
42	R.grandiflora	0.7145	0.7349	0.6518	0.7246	0.6173	0.7428	0.6857	0.7996	0.8329	0.8151	0.8274	0.6254
43	R.hectori	0.7311	0.7670	0.6881	0.7322	0.7012	0.7948	0.7054	0.8235	0.8350	0.8293	0.8407	0.7233
44	R.hookeri	0.6709	0.6897	0.7075	0.6522	0.6186	0.6999	0.6041	0.7466	0.7586	0.7756	0.7643	0.6484
45	R."L"	0.7274	0.7623	0.6798	0.7314	0.7282	0.7843	0.6912	0.8207	0.8297	0.8225	0.8354	0.7243
46	R."M"	0.7257	0.8334	0.7386	0.7167	0.7187	0.8473	0.6842	0.7412	0.7203	0.7133	0.7259	0.6911
47	R.petriensis	0.8260	0.7398	0.6238	0.8081	0.6222	0.7302	0.7771	0.7969	0.7938	0.8207	0.7881	0.6595
48	R.tenuicaulis	0.7227	0.7666	0.6587	0.7044	0.6972	0.7732	0.6626	0.8189	0.8306	0.8473	0.8362	0.7246
49	Genus"Z"	0.6770	0.7195	0.5963	0.6587	0.5735	0.7324	0.6368	0.7631	0.7350	0.7058	0.7406	0.7103

	OTU no. ->	37	38	39	40	41	42	43	44	45	46	47	48
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	G.umbricola												
19	Ha.pulvinaris												
20	Ha.sinclairii												
21	He.backhousii												
22	He.bellidioides												
23	He.coralloides												
24	He.depressum												
25	He.dimorphums												
26	He.dimorphumn												
27	He.filicaule												
28	He.intermedium												
29	He.lanceolatum												
30	He.obcordatum												
31	He.parvifolium												
32	L.grandiceps												
33	L.leontopodium												
34	L."Marlborough"												
35	L."Peel"												
36	Pseudognaphalium												
37	Pterygopappus												
38	R.bryoides	0.8033											
39	R.cinerea	0.7612	0.8106										
40	R.eximia	0.8033	1.0000	0.8106									
41	R.glabra	0.8211	0.8771	0.8225	0.8771								
42	R.grandiflora	0.8400	0.8658	0.7559	0.8658	0.8112							
43	R.hectori	0.8033	1.0000	0.8290	1.0000	0.8744	0.8851						
44	R.hookeri	0.7485	0.8196	0.7701	0.8196	0.8607	0.7583	0.8411					
45	R."L"	0.8234	1.0000	0.8291	1.0000	0.8970	0.8599	1.0000	0.8386				
46	R."M"	0.7807	0.7930	0.7536	0.7930	0.8365	0.7690	0.8105	0.7266	0.8111			
47	R.petriensis	0.8444	0.8181	0.7598	0.8181	0.8391	0.8276	0.8388	0.7902	0.8372	0.7993		
48	R.tenuicaulis	0.8278	0.8903	0.8419	0.8903	0.9305	0.8304	0.9146	0.9318	0.9105	0.7993	0.8632	
49	Genus"Z"	0.7223	0.7678	0.7830	0.7678	0.7959	0.7242	0.7802	0.7717	0.7852	0.7062	0.7273	0.7990

	OTU no. ->	1	2	3	4	5	6	7	8	9	10	11	12
1	A.keriensis												
2	A.rupestris	0.9737											
3	A.subrigida	0.9211	0.9474										
4	A.trinervis	0.9474	0.9211	0.9737									
5	A.triplinervis	0.7105	0.6842	0.7368	0.7632								
6	C.aculeata	0.7105	0.6842	0.7368	0.7632	0.7368							
7	C.fulvida	0.6757	0.6486	0.7027	0.7297	0.7027	0.7838						
8	C.leptophylla	0.6757	0.6486	0.7027	0.7297	0.7027	0.7838	1.0000					
9	C.longifolia	0.7368	0.7105	0.7632	0.7895	0.7632	0.9737	0.7568	0.7568				
10	E.catipes	0.7632	0.7368	0.7368	0.7632	0.8421	0.6842	0.6486	0.6486	0.7105			
11	E.meredithae	0.7632	0.7368	0.7368	0.7632	0.8421	0.6842	0.6486	0.6486	0.7105	0.8947		
12	E.planchonii	0.7368	0.7105	0.7632	0.7895	0.8684	0.7105	0.6757	0.6757	0.7368	0.9211	0.8684	
13	E.sinclairii	0.8684	0.8421	0.8421	0.8684	0.7895	0.7368	0.7027	0.7027	0.7632	0.8421	0.8421	0.8158
14	G.involucratum	0.7368	0.7105	0.7632	0.7895	0.8684	0.7105	0.6757	0.6757	0.7368	0.9211	0.8684	0.9474
15	G.mackayi	0.7368	0.7105	0.7632	0.7895	0.8684	0.7105	0.6757	0.6757	0.7368	0.9211	0.8684	1.0000
16	G.nitidulum	0.7895	0.7632	0.8158	0.8421	0.8684	0.7632	0.7297	0.7297	0.7895	0.8684	0.8684	0.8947
17	G.traversii	0.8919	0.8649	0.9189	0.9459	0.7568	0.7568	0.7222	0.7222	0.7838	0.7568	0.7568	0.7838
18	Ha.pulvinaris	0.8421	0.8158	0.8684	0.8947	0.7632	0.7632	0.7297	0.7297	0.7895	0.7632	0.7632	0.7895
19	Ha.sinclairii	0.8108	0.7838	0.8378	0.8649	0.7838	0.7297	0.7500	0.7500	0.7568	0.7838	0.7297	0.7568
20	He.backhousii	0.7632	0.7368	0.7895	0.8158	0.7895	0.7895	0.9189	0.9189	0.8158	0.7368	0.7368	0.7632
21	He.bellidioides	1.0000	0.9737	0.9211	0.9474	0.7105	0.7105	0.6757	0.6757	0.7368	0.7632	0.7632	0.7368
22	He.coralloides	0.9143	0.8857	0.9143	0.9429	0.8571	0.8000	0.7647	0.7647	0.8286	0.8857	0.8857	0.8857
23	He.depressum	0.8378	0.8108	0.8649	0.8919	0.8649	0.7568	0.7222	0.7222	0.7838	0.8649	0.8649	0.9189
24	He.dimorphum	0.8947	0.8684	0.9211	0.9474	0.8158	0.8158	0.7838	0.7838	0.8421	0.8158	0.8158	0.8421
25	He.filicaule	0.9737	0.9474	0.9474	0.9737	0.7368	0.7368	0.7027	0.7027	0.7632	0.7895	0.7895	0.7632
26	He.intermedium	0.8947	0.8684	0.9211	0.9474	0.8158	0.7632	0.7297	0.7297	0.7895	0.8158	0.8158	0.8421
27	He.lanceolatum	0.8378	0.8108	0.8649	0.8919	0.8108	0.8919	0.8378	0.8378	0.9189	0.8108	0.8108	0.8378
28	He.obcordatum	0.6316	0.6053	0.6579	0.6842	0.7105	0.7632	0.9189	0.9189	0.7368	0.7105	0.7105	0.7368
29	He.parvifolium	0.8421	0.8158	0.8684	0.8947	0.7632	0.7105	0.6757	0.6757	0.7368	0.7632	0.8158	0.7895
30	L.grandiceps	0.7105	0.6842	0.6842	0.7105	0.6842	0.5263	0.4865	0.4865	0.5526	0.7368	0.7368	0.8158
31	L.leontopodium	0.5278	0.5000	0.5556	0.5833	0.6389	0.4722	0.4286	0.4286	0.5000	0.5833	0.5833	0.6111
32	L."Marlborough"	0.7632	0.7368	0.7895	0.8158	0.6842	0.6842	0.5946	0.5946	0.7105	0.6842	0.6316	0.6579
33	L."Peel"	0.7632	0.7368	0.7895	0.8158	0.6842	0.6842	0.5946	0.5946	0.7105	0.6842	0.6316	0.6579
34	Pseudognaphalium	0.7632	0.7368	0.7895	0.8158	0.8421	0.7368	0.7027	0.7027	0.7632	0.8421	0.8421	0.8684
35	Pterygopappus	0.7568	0.7297	0.7838	0.8108	0.7297	0.8108	0.7568	0.7568	0.8378	0.6757	0.7297	0.7027
36	R.bryoides	0.7895	0.7632	0.8158	0.8421	0.8158	0.8158	0.7297	0.7297	0.8421	0.7632	0.8158	0.8421
37	R.cinerea	0.8684	0.8421	0.8421	0.8684	0.7368	0.7368	0.7027	0.7027	0.7632	0.7895	0.7895	0.8158
38	R.eximia	0.8684	0.8421	0.8947	0.9211	0.7368	0.7368	0.7027	0.7027	0.7632	0.7368	0.7895	0.7632
39	R.glabra	0.7838	0.7568	0.7838	0.8108	0.7838	0.6757	0.6389	0.6389	0.7027	0.8649	0.8108	0.9189
40	R.grandiflora	0.6316	0.6053	0.6579	0.6842	0.8158	0.6579	0.5676	0.5676	0.6842	0.8158	0.7632	0.8947
41	R.hectori	0.8919	0.8649	0.8378	0.8649	0.8378	0.7297	0.6389	0.6389	0.7568	0.8108	0.8108	0.8108
42	R.hookeri	0.7105	0.6842	0.7368	0.7632	0.7368	0.5789	0.5405	0.5405	0.6053	0.7895	0.7368	0.8158
43	R."L"	0.7368	0.7105	0.7632	0.7895	0.8158	0.8158	0.7297	0.7297	0.8421	0.7632	0.7632	0.8421
44	R."M"	0.8684	0.8421	0.8947	0.9211	0.7895	0.7895	0.7568	0.7568	0.8158	0.7895	0.7895	0.8158
45	R.petriensis	0.8108	0.7838	0.8378	0.8649	0.8108	0.6757	0.6389	0.6389	0.7027	0.8649	0.8108	0.9459
46	R.tenuicaulis	0.7368	0.7105	0.7105	0.7368	0.7105	0.5526	0.5135	0.5135	0.5789	0.7632	0.7105	0.7895
47	Genus "Z"	0.8333	0.8056	0.8611	0.8889	0.7500	0.6389	0.6000	0.6000	0.6667	0.8056	0.7500	0.8333

	OTU no. ->	13	14	15	16	17	18	19	20	21	22	23	24
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum	0.8158											
15	G.mackayi	0.8158	0.9474										
16	G.nitidulum	0.8684	0.8947	0.8947									
17	G.traversii	0.8919	0.7838	0.7838	0.8378								
18	Ha.pulvinaris	0.8158	0.7895	0.7895	0.8421	0.9459							
19	Ha.sinclairii	0.7838	0.7568	0.7568	0.8108	0.8889	0.9459						
20	He.backhousii	0.7895	0.7632	0.7632	0.8158	0.8108	0.8158	0.8378					
21	He.bellidioides	0.8684	0.7368	0.7368	0.7895	0.8919	0.8421	0.8108	0.7632				
22	He.coralloides	0.9143	0.8857	0.8857	0.8857	0.9412	0.9143	0.8824	0.8571	0.9143			
23	He.depressum	0.8108	0.8919	0.9189	0.8919	0.8889	0.8919	0.8611	0.8108	0.8378	0.9706		
24	He.dimorphum	0.8684	0.8421	0.8421	0.8947	0.9459	0.9474	0.9189	0.8684	0.8947	0.9714	0.9459	
25	He.filicaule	0.8947	0.7632	0.7632	0.8158	0.9189	0.8684	0.8378	0.7895	0.9737	0.9429	0.8649	0.9211
26	He.intermedium	0.8684	0.8421	0.8421	0.8421	0.9459	0.8947	0.8649	0.8158	0.8947	1.0000	0.9459	0.9474
27	He.lanceolatum	0.8649	0.8378	0.8378	0.8919	0.8889	0.8919	0.8611	0.9189	0.8378	0.9412	0.8889	0.9459
28	He.obcordatum	0.6579	0.7368	0.7368	0.7368	0.6757	0.6842	0.6486	0.8158	0.6316	0.7714	0.7838	0.7368
29	He.parvifolium	0.8158	0.7895	0.7895	0.7895	0.8919	0.8421	0.8108	0.7632	0.8421	0.9429	0.8919	0.8947
30	L.grandiceps	0.7368	0.7632	0.8158	0.7105	0.7297	0.6579	0.5946	0.5789	0.7105	0.7143	0.7297	0.6579
31	L.leontopodium	0.5833	0.6111	0.6111	0.5556	0.6000	0.5278	0.5833	0.5278	0.5278	0.6061	0.6286	0.5556
32	L."Marlborough"	0.7368	0.7105	0.6579	0.7105	0.8108	0.7632	0.7568	0.6842	0.7632	0.7714	0.7027	0.7632
33	L."Peel"	0.7368	0.7105	0.6579	0.7105	0.8108	0.7632	0.7568	0.6842	0.7632	0.7714	0.7027	0.7632
34	Pseudognaphalium	0.8947	0.8684	0.8684	0.8684	0.8378	0.8158	0.7838	0.7895	0.7632	0.9143	0.8649	0.8684
35	Pterygopappus	0.7297	0.7027	0.7027	0.7568	0.8056	0.8108	0.8333	0.8378	0.7568	0.8235	0.8056	0.8649
36	R.bryoides	0.8158	0.7895	0.8421	0.8421	0.8378	0.8421	0.8108	0.8158	0.7895	0.8857	0.8649	0.8947
37	R.cinerea	0.8947	0.7632	0.8158	0.8158	0.9189	0.8684	0.8108	0.7895	0.8684	0.9143	0.8378	0.8684
38	R.eximia	0.7895	0.7632	0.7632	0.8158	0.9189	0.9211	0.8649	0.7895	0.8684	0.8857	0.8649	0.9211
39	R.glabra	0.8108	0.8649	0.9189	0.8108	0.8333	0.8108	0.7778	0.7297	0.7838	0.8857	0.9444	0.8649
40	R.grandiflora	0.7105	0.8947	0.8947	0.7895	0.6757	0.6842	0.6486	0.6579	0.6316	0.7714	0.8108	0.7368
41	R.hectori	0.8108	0.8108	0.8108	0.8108	0.8056	0.7568	0.7222	0.7297	0.8919	0.8857	0.8611	0.8108
42	R.hookeri	0.7368	0.8684	0.8158	0.7632	0.7568	0.7105	0.6486	0.6316	0.7105	0.7714	0.7568	0.7105
43	R."L"	0.7632	0.7895	0.8421	0.8421	0.7838	0.7895	0.7568	0.8158	0.7368	0.8286	0.8649	0.8421
44	R."M"	0.8947	0.8158	0.8158	0.8684	0.9730	0.9211	0.8649	0.8421	0.8684	0.9429	0.8649	0.9211
45	R.petriensis	0.8108	0.8919	0.9459	0.8378	0.8056	0.7568	0.7222	0.7297	0.8108	0.8824	0.8889	0.8108
46	R.tenuicaulis	0.7105	0.8421	0.7895	0.7368	0.7297	0.6842	0.6216	0.6053	0.7368	0.7429	0.7297	0.6842
47	Genus "Z"	0.8056	0.8611	0.8333	0.8333	0.8857	0.8333	0.7714	0.6944	0.8333	0.8182	0.8286	0.8333

	OTU no. ->	25	26	27	28	29	30	31	32	33	34	35	36
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	Ha.pulvinaris												
19	Ha.sinclairii												
20	He.backhousii												
21	He.bellidioides												
22	He.coralloides												
23	He.depressum												
24	He.dimorphum												
25	He.filicaule												
26	He.intermedium	0.9211											
27	He.lanceolatum	0.8649	0.8919										
28	He.obcordatum	0.6579	0.7368	0.8108									
29	He.parvifolium	0.8684	0.9474	0.8378	0.6842								
30	L.grandiceps	0.6842	0.7105	0.6486	0.5526	0.6579							
31	L.leontopodium	0.5556	0.6111	0.5429	0.4444	0.5556	0.6944						
32	L."Marlborough"	0.7895	0.7632	0.7568	0.5526	0.7105	0.6316	0.7500					
33	L."Peel"	0.7895	0.7632	0.7568	0.5526	0.7105	0.6316	0.7500	1.0000				
34	Pseudognaphalium	0.7895	0.8684	0.8649	0.7105	0.8684	0.7368	0.5833	0.6842	0.6842			
35	Pterygopappus	0.7838	0.8108	0.8649	0.6757	0.8108	0.5135	0.4571	0.6216	0.6216	0.7297		
36	R.bryoides	0.8158	0.8421	0.8919	0.6842	0.8421	0.6579	0.6111	0.7632	0.7632	0.8158	0.8108	
37	R.cinerea	0.8947	0.8684	0.8649	0.6579	0.8158	0.7368	0.6111	0.7895	0.7895	0.7895	0.7297	0.8684
38	R.eximia	0.8947	0.8684	0.8649	0.6579	0.8684	0.6842	0.5278	0.7895	0.7895	0.7895	0.8378	0.8684
39	R.glabra	0.8108	0.8649	0.8056	0.7027	0.8108	0.7838	0.6286	0.6216	0.6216	0.8378	0.7222	0.8108
40	R.grandiflora	0.6579	0.7368	0.7297	0.6316	0.6842	0.7632	0.7222	0.6579	0.6579	0.7632	0.5946	0.7895
41	R.hectori	0.8649	0.8649	0.8056	0.7027	0.8108	0.7838	0.6571	0.7838	0.7838	0.7838	0.6667	0.8108
42	R.hookeri	0.7368	0.7632	0.7027	0.6053	0.7105	0.7895	0.6944	0.7368	0.7368	0.7368	0.5676	0.6579
43	R."L"	0.7632	0.7895	0.8919	0.7368	0.7368	0.6579	0.6667	0.7105	0.7105	0.7632	0.7568	0.8947
44	R."M"	0.8947	0.9211	0.9189	0.7105	0.8684	0.7368	0.6111	0.8421	0.8421	0.8421	0.7838	0.8684
45	R.petriensis	0.8378	0.8649	0.8056	0.7027	0.8108	0.8649	0.6571	0.7297	0.7297	0.8108	0.6667	0.8108
46	R.tenuicaulis	0.7105	0.7368	0.6757	0.5789	0.6842	0.8158	0.6667	0.7105	0.7105	0.7105	0.5405	0.6316
47	Genus "Z"	0.8611	0.8333	0.7714	0.6111	0.7778	0.8056	0.5882	0.7778	0.7778	0.7500	0.6857	0.7222

	OTU no. ->	37	38	39	40	41	42	43	44	45	46	47
1	A.keriensis											
2	A.rupestris											
3	A.subrigida											
4	A.trinervis											
5	A.triplinervis											
6	C.aculeata											
7	C.fulvida											
8	C.leptophylla											
9	C.longifolia											
10	E.catipes											
11	E.meredithae											
12	E.planchonii											
13	E.sinclairii											
14	G.involucratum											
15	G.mackayi											
16	G.nitidulum											
17	G.traversii											
18	Ha.pulvinaris											
19	Ha.sinclairii											
20	He.backhousii											
21	He.bellidioides											
22	He.coralloides											
23	He.depressum											
24	He.dimorphum											
25	He.filicaule											
26	He.intermedium											
27	He.lanceolatum											
28	He.obcordatum											
29	He.parvifolium											
30	L.grandiceps											
31	L.leontopodium											
32	L."Marlborough"											
33	L."Peel"											
34	Pseudognaphalium											
35	Pterygopappus											
36	R.bryoides											
37	R.cinerea											
38	R.eximia	0.8421										
39	R.glabra	0.8108	0.7838									
40	R.grandiflora	0.7105	0.6579	0.8108								
41	R.hectori	0.8108	0.7838	0.7838	0.7568							
42	R.hookeri	0.7368	0.7368	0.7297	0.8158	0.7838						
43	R."L"	0.8158	0.7632	0.8108	0.8421	0.8108	0.7105					
44	R."M"	0.9474	0.8947	0.7838	0.7105	0.8378	0.7895	0.8158				
45	R.petriensis	0.8378	0.7838	0.8889	0.8378	0.8889	0.8649	0.8108	0.8378			
46	R.tenuicaulis	0.7105	0.7105	0.7027	0.7895	0.8108	0.9737	0.6842	0.7632	0.8378		
47	Genus "Z"	0.8056	0.8611	0.8000	0.7500	0.8000	0.9167	0.7222	0.8611	0.8857	0.8889	

	OTU no. ->	1	2	3	4	5	6	7	8	9	10	11	12
1	A.keriensis												
2	A.rupestris	0.8333											
3	A.subrigida	0.5000	0.6667										
4	A.trinervis	0.6000	0.5000	0.7500									
5	A.triplinervis	0.0000	0.0000	0.0000	0.0000								
6	C.aculeata	0.0000	0.0000	0.0000	0.0000	0.0909							
7	C.fulvida	0.0000	0.0000	0.0000	0.0000	0.0833	0.2000						
8	C.leptophylla	0.0000	0.0000	0.0000	0.0000	0.0833	0.2000	1.0000					
9	C.longifolia	0.0000	0.0000	0.0000	0.0000	0.1000	0.8333	0.1000	0.1000				
10	E.catipes	0.1000	0.0909	0.0000	0.0000	0.3333	0.0000	0.0000	0.0000	0.0000			
11	E.meredithae	0.1000	0.0909	0.0000	0.0000	0.3333	0.0000	0.0000	0.0000	0.0000	0.5000		
12	E.planchonii	0.0000	0.0000	0.0000	0.0000	0.3750	0.0000	0.0000	0.0000	0.0000	0.5714	0.3750	
13	E.sinclairii	0.2857	0.2500	0.1429	0.1667	0.1111	0.0000	0.0000	0.0000	0.0000	0.2500	0.2500	0.1250
14	G.involucratum	0.0000	0.0000	0.0000	0.0000	0.3750	0.0000	0.0000	0.0000	0.0000	0.5714	0.3750	0.6667
15	G.mackayi	0.0000	0.0000	0.0000	0.0000	0.3750	0.0000	0.0000	0.0000	0.0000	0.5714	0.3750	1.0000
16	G.nitidulum	0.0000	0.0000	0.0000	0.0000	0.2857	0.0000	0.0000	0.0000	0.0000	0.2857	0.2857	0.3333
17	G.traversii	0.3333	0.2857	0.4000	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
18	Ha.pulvinaris	0.1429	0.1250	0.1667	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
19	Ha.sinclairii	0.1250	0.1111	0.1429	0.1667	0.1111	0.0000	0.1000	0.1000	0.0000	0.1111	0.0000	0.0000
20	He.backhousii	0.0000	0.0000	0.0000	0.0000	0.1111	0.1111	0.5714	0.5714	0.1250	0.0000	0.0000	0.0000
21	He.bellidioides	1.0000	0.8333	0.5000	0.6000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.1000	0.0000
22	He.coralloides	0.0000	0.0000	0.0000	0.0000	0.1667	0.0000	0.0000	0.0000	0.0000	0.2000	0.2000	0.2000
23	He.depressum	0.1429	0.1250	0.1667	0.2000	0.2857	0.0000	0.0000	0.0000	0.0000	0.2857	0.2857	0.4000
24	He.dimorphum	0.2000	0.1667	0.2500	0.3333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	He.filicaule	0.8000	0.6667	0.6000	0.7500	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111	0.1111	0.0000
26	He.intermedium	0.3333	0.2857	0.4000	0.5000	0.1250	0.0000	0.0000	0.0000	0.0000	0.1250	0.1250	0.1429
27	He.lanceolatum	0.0000	0.0000	0.0000	0.0000	0.0000	0.2000	0.1429	0.1429	0.2500	0.0000	0.0000	0.0000
28	He.obcordatum	0.0000	0.0000	0.0000	0.0000	0.1538	0.2500	0.6667	0.6667	0.1667	0.1538	0.1538	0.1667
29	He.parvifolium	0.2500	0.2222	0.2857	0.3333	0.1000	0.0000	0.0000	0.0000	0.0000	0.1000	0.2222	0.1111
30	L.grandiceps	0.2143	0.2000	0.1429	0.1538	0.2000	0.0000	0.0000	0.0000	0.0000	0.2857	0.2857	0.4167
31	L.leontopodium	0.0556	0.0526	0.0588	0.0625	0.2353	0.0500	0.0476	0.0476	0.0526	0.1667	0.1667	0.1765
32	L."Marlborough"	0.1818	0.1667	0.2000	0.2222	0.0769	0.0769	0.0000	0.0000	0.0833	0.0769	0.0000	0.0000
33	L."Peel"	0.1818	0.1667	0.2000	0.2222	0.0769	0.0769	0.0000	0.0000	0.0833	0.0769	0.0000	0.0000
34	Pseudognaphalium	0.0000	0.0000	0.0000	0.0000	0.2500	0.0000	0.0000	0.0000	0.0000	0.2500	0.2500	0.2857
35	Pterygopappus	0.1000	0.0909	0.1111	0.1250	0.0909	0.2222	0.1818	0.1818	0.2500	0.0000	0.0909	0.0000
36	R.bryoides	0.0000	0.0000	0.0000	0.0000	0.1250	0.1250	0.0000	0.0000	0.1429	0.0000	0.1250	0.1429
37	R.cinerea	0.2857	0.2500	0.1429	0.1667	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111	0.1111	0.1250
38	R.eximia	0.2857	0.2500	0.3333	0.4000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111	0.0000
39	R.glabra	0.1111	0.1000	0.1111	0.1250	0.2000	0.0000	0.0000	0.0000	0.0000	0.3750	0.2222	0.5714
40	R.grandiflora	0.0000	0.0000	0.0000	0.0000	0.3636	0.0714	0.0000	0.0000	0.0769	0.3636	0.2500	0.5556
41	R.hectori	0.4286	0.3750	0.2500	0.2857	0.3333	0.0909	0.0000	0.0000	0.1000	0.2222	0.2222	0.2222
42	R.hookeri	0.1538	0.1429	0.1667	0.1818	0.2308	0.0000	0.0000	0.0000	0.0000	0.3333	0.2308	0.3636
43	R."L"	0.0000	0.0000	0.0000	0.0000	0.2222	0.2222	0.0909	0.0909	0.2500	0.1000	0.1000	0.2500
44	R."M"	0.1667	0.1429	0.2000	0.2500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
45	R.petriensis	0.2222	0.2000	0.2500	0.2857	0.2222	0.0000	0.0000	0.0000	0.0000	0.3750	0.2222	0.6667
46	R.tenuicaulis	0.2308	0.2143	0.1538	0.1667	0.2143	0.0000	0.0000	0.0000	0.0000	0.3077	0.2143	0.3333
47	Genus "Z"	0.3333	0.3000	0.3750	0.4286	0.1818	0.0000	0.0000	0.0000	0.0000	0.3000	0.1818	0.3333

	OTU no. ->	13	14	15	16	17	18	19	20	21	22	23	24
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum	0.1250											
15	G.mackayi	0.1250	0.6667										
16	G.nitidulum	0.1667	0.3333	0.3333									
17	G.traversii	0.2000	0.0000	0.0000	0.0000								
18	Ha.pulvinaris	0.0000	0.0000	0.0000	0.0000	0.5000							
19	Ha.sinclairii	0.0000	0.0000	0.0000	0.0000	0.2000	0.5000						
20	He.backhousii	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1429					
21	He.bellidioides	0.2857	0.0000	0.0000	0.0000	0.3333	0.1429	0.1250	0.0000				
22	He.coralloides	0.0000	0.2000	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
23	He.depressum	0.0000	0.3333	0.4000	0.2000	0.2000	0.2000	0.1667	0.0000	0.1429	0.5000		
24	He.dimorphum	0.0000	0.0000	0.0000	0.0000	0.3333	0.3333	0.2500	0.0000	0.2000	0.0000	0.3333	
25	He.filicaule	0.3333	0.0000	0.0000	0.0000	0.4000	0.1667	0.1429	0.0000	0.8000	0.0000	0.1667	0.2500
26	He.intermedium	0.1667	0.1429	0.1429	0.0000	0.5000	0.2000	0.1667	0.0000	0.3333	1.0000	0.5000	0.3333
27	He.lanceolatum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2500	0.0000	0.0000	0.0000	0.0000
28	He.obcordatum	0.0000	0.1667	0.1667	0.0909	0.0000	0.0000	0.0000	0.3000	0.0000	0.1111	0.2000	0.0000
29	He.parvifolium	0.1250	0.1111	0.1111	0.0000	0.3333	0.1429	0.1250	0.0000	0.2500	0.3333	0.3333	0.2000
30	L.grandiceps	0.2308	0.3077	0.4167	0.1538	0.1667	0.0714	0.0000	0.0000	0.2143	0.0909	0.1667	0.0000
31	L.leontopodium	0.1176	0.1765	0.1765	0.0588	0.0667	0.0000	0.1176	0.0556	0.0556	0.0714	0.1333	0.0000
32	L."Marlborough"	0.0909	0.0833	0.0000	0.0000	0.2222	0.1000	0.1000	0.0000	0.1818	0.0000	0.0000	0.0000
33	L."Peel"	0.0909	0.0833	0.0000	0.0000	0.2222	0.1000	0.1000	0.0000	0.1818	0.0000	0.0000	0.0000
34	Pseudognaphalium	0.3333	0.2857	0.2857	0.1667	0.0000	0.0000	0.0000	0.0000	0.0000	0.2500	0.1667	0.0000
35	Pterygopappus	0.0000	0.0000	0.0000	0.0000	0.1250	0.1250	0.2500	0.2500	0.1000	0.0000	0.1250	0.1667
36	R.bryoides	0.0000	0.0000	0.1429	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
37	R.cinerea	0.3333	0.0000	0.1250	0.0000	0.4000	0.1667	0.0000	0.0000	0.2857	0.0000	0.0000	0.0000
38	R.eximia	0.0000	0.0000	0.0000	0.0000	0.4000	0.4000	0.1667	0.0000	0.2857	0.0000	0.1667	0.2500
39	R.glabra	0.1250	0.3750	0.5714	0.1250	0.1429	0.1250	0.1111	0.0000	0.1111	0.2000	0.6000	0.1667
40	R.grandiflora	0.0833	0.5556	0.5556	0.2000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111	0.2222	0.0000
41	R.hectori	0.1250	0.2222	0.2222	0.1250	0.1250	0.0000	0.0000	0.0000	0.4286	0.2000	0.2857	0.0000
42	R.hookeri	0.1667	0.5000	0.3636	0.1818	0.1818	0.0833	0.0000	0.0000	0.1538	0.1111	0.1818	0.0000
43	R."L"	0.0000	0.1111	0.2500	0.1429	0.0000	0.0000	0.0000	0.1250	0.0000	0.0000	0.1667	0.0000
44	R."M"	0.2000	0.0000	0.0000	0.0000	0.6667	0.2500	0.0000	0.0000	0.1667	0.0000	0.0000	0.0000
45	R.petriensis	0.1250	0.4286	0.6667	0.1429	0.1250	0.0000	0.0000	0.0000	0.2222	0.2000	0.3333	0.0000
46	R.tenuicaulis	0.1538	0.4545	0.3333	0.1667	0.1667	0.0769	0.0000	0.0000	0.2308	0.1000	0.1667	0.0000
47	Genus "Z"	0.2222	0.3750	0.3333	0.2500	0.4286	0.2500	0.1111	0.0000	0.3333	0.0000	0.2500	0.1429

	OTU no. ->	25	26	27	28	29	30	31	32	33	34	35	36
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	Ha.pulvinaris												
19	Ha.sinclairii												
20	He.backhousii												
21	He.bellidioides												
22	He.coralloides												
23	He.depressum												
24	He.dimorphum												
25	He.filicaule												
26	He.intermedium	0.4000											
27	He.lanceolatum	0.0000	0.0000										
28	He.obcordatum	0.0000	0.0909	0.1250									
29	He.parvifolium	0.2857	0.6000	0.0000	0.0769								
30	L.grandiceps	0.1429	0.1538	0.0000	0.1053	0.1333							
31	L.leontopodium	0.0588	0.1250	0.0000	0.0909	0.1111	0.3889						
32	L."Marlborough"	0.2000	0.1000	0.0000	0.0000	0.0833	0.1765	0.4000					
33	L."Peel"	0.2000	0.1000	0.0000	0.0000	0.0833	0.1765	0.4000	1.0000				
34	Pseudognaphalium	0.0000	0.1667	0.0000	0.0833	0.2857	0.2308	0.1176	0.0000	0.0000			
35	Pterygopappus	0.1111	0.1250	0.1667	0.0769	0.2222	0.0000	0.0500	0.0000	0.0000	0.0000		
36	R.bryoides	0.0000	0.0000	0.0000	0.0000	0.1429	0.0714	0.1250	0.1000	0.1000	0.0000	0.1250	
37	R.cinerea	0.3333	0.1667	0.0000	0.0000	0.1250	0.2308	0.1250	0.2000	0.2000	0.0000	0.0000	0.1667
38	R.eximia	0.3333	0.1667	0.0000	0.0000	0.2857	0.1429	0.0000	0.2000	0.2000	0.0000	0.2500	0.1667
39	R.glabra	0.1250	0.2857	0.0000	0.1538	0.2222	0.3846	0.2353	0.0000	0.0000	0.2500	0.0909	0.1250
40	R.grandiflora	0.0000	0.0909	0.0000	0.1250	0.0769	0.4000	0.4118	0.1333	0.1333	0.1818	0.0000	0.2000
41	R.hectori	0.2857	0.2857	0.0000	0.1538	0.2222	0.3846	0.2500	0.2727	0.2727	0.1111	0.0000	0.1250
42	R.hookeri	0.1667	0.1818	0.0000	0.1176	0.1538	0.4667	0.3529	0.2857	0.2857	0.1667	0.0000	0.0000
43	R."L"	0.0000	0.0000	0.2000	0.1667	0.0000	0.1333	0.2500	0.0833	0.0833	0.0000	0.1000	0.3333
44	R."M"	0.2000	0.2500	0.0000	0.0000	0.1667	0.1667	0.0667	0.2500	0.2500	0.0000	0.0000	0.0000
45	R.petriensis	0.2500	0.2857	0.0000	0.1538	0.2222	0.5455	0.2500	0.1667	0.1667	0.1250	0.0000	0.1250
46	R.tenuicaulis	0.1538	0.1667	0.0000	0.1111	0.1429	0.5333	0.3333	0.2667	0.2667	0.1538	0.0000	0.0000
47	Genus "Z"	0.3750	0.2500	0.0000	0.0667	0.2000	0.4615	0.1250	0.2727	0.2727	0.1000	0.0833	0.0000

	OTU no. ->	37	38	39	40	41	42	43	44	45	46	47
1	A.keriensis											
2	A.rupestris											
3	A.subrigida											
4	A.trinervis											
5	A.triplinervis											
6	C.aculeata											
7	C.fulvida											
8	C.leptophylla											
9	C.longifolia											
10	E.catipes											
11	E.meredithae											
12	E.planchonii											
13	E.sinclairii											
14	G.involucratum											
15	G.mackayi											
16	G.nitidulum											
17	G.traversii											
18	Ha.pulvinaris											
19	Ha.sinclairii											
20	He.backhousii											
21	He.bellidioides											
22	He.coralloides											
23	He.depressum											
24	He.dimorphum											
25	He.filicaule											
26	He.intermedium											
27	He.lanceolatum											
28	He.obcordatum											
29	He.parvifolium											
30	L.grandiceps											
31	L.leontopodium											
32	L."Marlborough"											
33	L."Peel"											
34	Pseudognaphalium											
35	Pterygopappus											
36	R.bryoides											
37	R.cinerea											
38	R.eximia	0.1429										
39	R.glabra	0.1250	0.1111									
40	R.grandiflora	0.0833	0.0000	0.3636								
41	R.hectori	0.1250	0.1111	0.2000	0.2500							
42	R.hookeri	0.1667	0.1667	0.2308	0.4615	0.3333						
43	R."L"	0.1250	0.0000	0.2222	0.4000	0.2222	0.1538					
44	R."M"	0.5000	0.2000	0.0000	0.0000	0.1429	0.2000	0.0000				
45	R.petriensis	0.2500	0.1111	0.5000	0.4000	0.5000	0.5000	0.2222	0.1429			
46	R.tenuicaulis	0.1538	0.1538	0.2143	0.4286	0.4167	0.9091	0.1429	0.1818	0.4545		
47	Genus "Z"	0.2222	0.3750	0.3000	0.2500	0.3000	0.6667	0.0909	0.2857	0.5000	0.6000	

Similarity matrix from combined data with Gower's coefficient (flavonoid data with simple matching coefficient)

	OTU no. ->	1	2	3	4	5	6	7	8	9	10	11	12
1	A.keriensis												
2	A.rupestris	0.9579											
3	A.subrigida	0.9416	0.9405										
4	A.trinervis	0.9581	0.9356	0.9611									
5	A.triplinervis	0.7464	0.7335	0.7610	0.7659								
6	C.aculeata	0.6933	0.6779	0.7016	0.7156	0.6788							
7	C.fulvida	0.6853	0.6689	0.6880	0.7024	0.7234	0.8139						
8	C.leptophylla	0.6829	0.6673	0.6904	0.7000	0.7250	0.8282	0.9857					
9	C.longifolia	0.6979	0.6825	0.7062	0.7202	0.7291	0.9366	0.8186	0.8210				
10	E.catipes	0.6898	0.6706	0.6658	0.6885	0.7251	0.7287	0.7478	0.7453	0.7578			
11	E.meredithae	0.7496	0.7459	0.7340	0.7462	0.7734	0.7965	0.7891	0.7916	0.8126	0.8710		
12	E.planchonii	0.7204	0.7017	0.7209	0.7430	0.7668	0.7780	0.7894	0.7870	0.8064	0.8786	0.9080	
13	E.sinclairii	0.7438	0.7280	0.7304	0.7370	0.7421	0.7439	0.7460	0.7460	0.8088	0.7895	0.8035	0.7768
14	G.involucratum	0.7423	0.7269	0.7521	0.7646	0.8763	0.7052	0.7431	0.7336	0.7555	0.7874	0.8074	0.8277
15	G.mackayi	0.6728	0.6538	0.6787	0.6901	0.7436	0.7264	0.7308	0.7283	0.7552	0.8545	0.8215	0.9064
16	G.nitidulum	0.7039	0.6844	0.7043	0.7273	0.7462	0.7672	0.7637	0.7612	0.8090	0.8362	0.8375	0.8744
17	G.traversii	0.7761	0.7602	0.7798	0.8114	0.7284	0.7343	0.7570	0.7554	0.7756	0.7758	0.7760	0.7841
18	Ha.pulvinaris	0.7577	0.7413	0.7745	0.7757	0.7671	0.7543	0.7299	0.7299	0.7710	0.7139	0.7530	0.7767
19	Ha.sinclairii	0.7886	0.7720	0.7941	0.8068	0.8050	0.7599	0.7608	0.7608	0.7767	0.7385	0.7585	0.7698
20	He.backhousii	0.7243	0.7080	0.7270	0.7412	0.7620	0.8161	0.9643	0.9500	0.8442	0.7874	0.8278	0.8277
21	He.bellidioides	0.8751	0.8476	0.8374	0.8619	0.7319	0.7448	0.7623	0.7647	0.7732	0.7966	0.8130	0.7889
22	He.coralloides	0.7193	0.7012	0.7179	0.7247	0.6838	0.7795	0.7508	0.7508	0.7969	0.8133	0.8169	0.8100
23	He.depressum	0.6852	0.6694	0.6991	0.7026	0.7134	0.7554	0.7408	0.7311	0.7641	0.7962	0.8072	0.8206
24	He.dimorphumn	0.7991	0.7834	0.8092	0.8220	0.7736	0.8220	0.8392	0.8295	0.8501	0.8156	0.8650	0.8683
25	He.dimorphums	0.7215	0.7061	0.7367	0.7385	0.6902	0.7743	0.7683	0.7588	0.7907	0.7823	0.7932	0.7946
26	He.filicaule	0.8015	0.7820	0.7772	0.8002	0.6824	0.6932	0.6776	0.6751	0.6973	0.7314	0.7266	0.7369
27	He.intermedium	0.7118	0.6941	0.7184	0.7288	0.6643	0.7537	0.7410	0.7267	0.7700	0.7723	0.7761	0.7848
28	He.lanceolatum	0.7616	0.7302	0.7499	0.7725	0.7429	0.7800	0.7645	0.7621	0.8092	0.7451	0.7481	0.7797
29	He.obcordatum	0.6803	0.6649	0.6901	0.7026	0.7324	0.8052	0.9065	0.9089	0.8098	0.7718	0.8151	0.8124
30	He.parvifolium	0.7034	0.6748	0.7193	0.7211	0.6593	0.7176	0.6856	0.6881	0.7471	0.7281	0.7656	0.7424
31	L.grandiceps	0.6575	0.6417	0.6411	0.6508	0.6211	0.6358	0.6315	0.6339	0.6767	0.7791	0.7533	0.7748
32	L.leontopodium	0.5456	0.5261	0.5516	0.5634	0.5690	0.5749	0.5775	0.5750	0.6168	0.6844	0.6437	0.6778
33	L."Marlborough"	0.6491	0.6301	0.6550	0.6664	0.5876	0.6657	0.6449	0.6425	0.6945	0.7256	0.6610	0.6939
34	L."Peel"	0.6559	0.6369	0.6618	0.6733	0.5944	0.6725	0.6518	0.6494	0.7134	0.7317	0.6680	0.7009
35	Pseudognaphalium	0.7278	0.7122	0.7446	0.7449	0.7694	0.7684	0.7793	0.7681	0.7955	0.7768	0.8245	0.8178
36	Pterygopappus	0.6589	0.6404	0.6703	0.6774	0.6540	0.7478	0.7629	0.7629	0.7785	0.7690	0.7575	0.7486
37	R.bryoides	0.6721	0.6531	0.6725	0.6949	0.6774	0.7839	0.7536	0.7511	0.8127	0.8517	0.8012	0.8317
38	R.cinerea	0.7490	0.7332	0.7348	0.7422	0.6938	0.7357	0.7385	0.7377	0.7886	0.7836	0.7809	0.8064
39	R.eximia	0.7083	0.6893	0.7087	0.7311	0.6413	0.7477	0.7414	0.7389	0.7765	0.8396	0.7889	0.7952
40	R.glabra	0.6943	0.6783	0.6913	0.6996	0.6963	0.7170	0.7177	0.7161	0.7462	0.8549	0.8005	0.8629
41	R.grandiflora	0.5923	0.5733	0.5927	0.6096	0.6699	0.6597	0.6605	0.6581	0.7006	0.8282	0.7531	0.8315
42	R.hectori	0.7223	0.7043	0.6903	0.7085	0.6937	0.7549	0.7202	0.7202	0.7848	0.8744	0.8107	0.8258
43	R.hookeri	0.6162	0.5969	0.6166	0.6337	0.6093	0.6600	0.6362	0.6337	0.6648	0.7947	0.7329	0.7846
44	R."L"	0.6559	0.6367	0.6564	0.6790	0.6857	0.7934	0.7629	0.7604	0.8226	0.8621	0.7863	0.8420
45	R."M"	0.7739	0.7584	0.7782	0.7962	0.7279	0.7783	0.7854	0.7846	0.8064	0.7983	0.8119	0.8357
46	R.petriensis	0.6786	0.6596	0.6790	0.6959	0.6653	0.6885	0.6869	0.6845	0.7173	0.8414	0.7798	0.8596
47	R.tenuicaulis	0.6569	0.6379	0.6332	0.6502	0.6381	0.6641	0.6650	0.6626	0.6929	0.8216	0.7576	0.8116
48	Genus "Z"	0.6750	0.6556	0.6867	0.6928	0.6679	0.6807	0.6846	0.6821	0.7473	0.7916	0.7489	0.7775

Similarity matrix from combined data with Gower's coefficient (flavonoid data with simple matching coefficient)

	OTU no. ->	13	14	15	16	17	18	19	20	21	22	23	24
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum	0.7686											
15	G.mackayi	0.8380	0.8023										
16	G.nitidulum	0.8552	0.7928	0.9185									
17	G.traversii	0.8711	0.7745	0.8405	0.8665								
18	Ha.pulvinaris	0.7883	0.7416	0.7776	0.8068	0.7983							
19	Ha.sinclairii	0.7885	0.7722	0.7615	0.7974	0.8145	0.9272						
20	He.backhousii	0.7852	0.7815	0.7702	0.8036	0.7966	0.7708	0.8018					
21	He.bellidioides	0.7761	0.7808	0.7319	0.7705	0.8480	0.7535	0.7847	0.8008				
22	He.coralloides	0.7569	0.7284	0.7553	0.7674	0.7696	0.7636	0.7634	0.7914	0.8162			
23	He.depressum	0.7108	0.7415	0.7781	0.7780	0.7580	0.7667	0.7536	0.7801	0.7811	0.9584		
24	He.dimorphumn	0.7925	0.8323	0.8053	0.8463	0.8869	0.8203	0.8524	0.8773	0.8881	0.8557	0.8670	
25	He.dimorphums	0.7393	0.7318	0.7528	0.7891	0.7938	0.7907	0.7909	0.8063	0.8179	0.9456	0.9533	0.9142
26	He.filicaule	0.7137	0.7177	0.7079	0.7563	0.7834	0.7735	0.7632	0.7186	0.8485	0.7888	0.7297	0.8007
27	He.intermedium	0.7334	0.7138	0.7319	0.7431	0.7703	0.7513	0.7510	0.7794	0.7986	1.0000	0.9453	0.8476
28	He.lanceolatum	0.8108	0.7564	0.8067	0.8374	0.7965	0.8123	0.8317	0.8011	0.7582	0.7425	0.7167	0.8178
29	He.obcordatum	0.7171	0.7658	0.7492	0.7631	0.7571	0.7134	0.7437	0.8606	0.7697	0.7643	0.7648	0.8331
30	He.parvifolium	0.7174	0.6939	0.6863	0.6968	0.7286	0.7390	0.7385	0.7265	0.7793	0.9740	0.9054	0.8052
31	L.grandiceps	0.7850	0.6780	0.8116	0.7660	0.7831	0.6454	0.6399	0.6721	0.7400	0.7254	0.7311	0.7031
32	L.leontopodium	0.6892	0.5797	0.7329	0.7001	0.7019	0.5791	0.6117	0.6197	0.6296	0.6671	0.6641	0.6283
33	L."Marlborough"	0.7411	0.6222	0.7476	0.7561	0.7794	0.6916	0.6906	0.6853	0.7323	0.7420	0.6994	0.7199
34	L."Peel"	0.7599	0.6290	0.7544	0.7692	0.7987	0.6925	0.6980	0.6921	0.7392	0.7356	0.6940	0.7269
35	Pseudognaphalium	0.8233	0.8194	0.8077	0.8118	0.7684	0.7864	0.7744	0.8177	0.7362	0.7922	0.7701	0.8258
36	Pterygopappus	0.7081	0.6613	0.7208	0.7589	0.7707	0.7253	0.7419	0.8013	0.7706	0.7802	0.7787	0.8166
37	R.bryoides	0.7707	0.6908	0.8262	0.8329	0.7757	0.7680	0.7544	0.7927	0.7774	0.8002	0.7846	0.8150
38	R.cinerea	0.8352	0.7283	0.8090	0.8167	0.8256	0.8002	0.7648	0.7778	0.8009	0.7727	0.7379	0.8029
39	R.eximia	0.7586	0.6788	0.7900	0.8205	0.8127	0.8065	0.7803	0.7806	0.8140	0.8002	0.7846	0.8273
40	R.glabra	0.7662	0.7556	0.8761	0.8227	0.8057	0.7633	0.7697	0.7578	0.8070	0.8042	0.8274	0.8330
41	R.grandiflora	0.6908	0.7340	0.8058	0.7870	0.7088	0.6510	0.6423	0.7008	0.6992	0.7531	0.7617	0.7358
42	R.hectori	0.7771	0.7121	0.8088	0.8277	0.7687	0.7347	0.7201	0.7612	0.8377	0.8002	0.7934	0.7878
43	R.hookeri	0.7037	0.6986	0.7835	0.7702	0.7097	0.7262	0.6731	0.6772	0.7123	0.7143	0.7008	0.6996
44	R."L"	0.7557	0.6992	0.8363	0.8433	0.7604	0.7520	0.7380	0.8023	0.7623	0.7820	0.7846	0.8002
45	R."M"	0.7865	0.7645	0.7728	0.8147	0.8525	0.7861	0.7921	0.8232	0.8699	0.8227	0.7870	0.8735
46	R.petriensis	0.7346	0.7214	0.8497	0.8184	0.7546	0.6954	0.6875	0.7268	0.7662	0.8518	0.8462	0.7722
47	R.tenuicaulis	0.7314	0.7264	0.8102	0.7977	0.7380	0.7006	0.6731	0.7052	0.7646	0.7308	0.7169	0.7280
48	Genus "Z"	0.7827	0.7404	0.7805	0.7972	0.7970	0.7556	0.7232	0.7255	0.7856	0.7326	0.7343	0.7714

Similarity matrix from combined data with Gower's coefficient (flavonoid data with simple matching coefficient)

	OTU no. ->	25	26	27	28	29	30	31	32	33	34	35	36
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	Ha.pulvinaris												
19	Ha.sinclairii												
20	He.backhousii												
21	He.bellidioides												
22	He.coralloides												
23	He.depressum												
24	He.dimorphumn												
25	He.dimorphums												
26	He.filicaule	0.7544											
27	He.intermedium	0.9323	0.7728										
28	He.lanceolatum	0.7455	0.7492	0.7192									
29	He.obcordatum	0.7466	0.6774	0.7354	0.7719								
30	He.parvifolium	0.8952	0.7322	0.9569	0.7107	0.7042							
31	L.grandiceps	0.7069	0.6269	0.7146	0.6587	0.6680	0.6749						
32	L.leontopodium	0.6286	0.5847	0.6597	0.6080	0.5859	0.6098	0.8246					
33	L."Marlborough"	0.7241	0.6928	0.7295	0.7013	0.6282	0.6844	0.7768	0.8742				
34	L."Peel"	0.7189	0.6982	0.7244	0.7084	0.6351	0.6789	0.7897	0.8872	0.9813			
35	Pseudognaphalium	0.7810	0.6918	0.7784	0.7577	0.7393	0.7562	0.7366	0.6375	0.6791	0.6860		
36	Pterygopappus	0.8171	0.7058	0.7758	0.7362	0.7350	0.7637	0.6734	0.6356	0.7123	0.7058	0.6941	
37	R.bryoides	0.7952	0.7399	0.7760	0.7948	0.7290	0.7581	0.7363	0.7257	0.7880	0.7947	0.7579	0.8069
38	R.cinerea	0.7618	0.7242	0.7486	0.7531	0.7103	0.7199	0.7723	0.7223	0.7975	0.7983	0.7744	0.7463
39	R.eximia	0.8073	0.7774	0.7879	0.7823	0.7170	0.7706	0.7483	0.6891	0.7999	0.8066	0.7457	0.8197
40	R.glabra	0.8006	0.7421	0.7881	0.7602	0.7618	0.7473	0.7991	0.7347	0.7251	0.7320	0.7779	0.7749
41	R.grandiflora	0.7248	0.6547	0.7301	0.6693	0.6919	0.6850	0.7829	0.7843	0.7440	0.7507	0.6892	0.7236
42	R.hectori	0.7680	0.7730	0.7928	0.7500	0.7522	0.7561	0.8051	0.7562	0.8085	0.8147	0.7516	0.7394
43	R.hookeri	0.6893	0.7216	0.7036	0.6580	0.6560	0.6560	0.7665	0.7297	0.7577	0.7516	0.6899	0.6616
44	R."L"	0.7805	0.7204	0.7583	0.8049	0.7623	0.7135	0.7453	0.7563	0.7706	0.7775	0.7425	0.7914
45	R."M"	0.8130	0.8119	0.8081	0.8090	0.7862	0.7706	0.7392	0.6718	0.7723	0.7791	0.7594	0.7822
46	R.petriensis	0.8192	0.7241	0.8334	0.7037	0.7180	0.7929	0.8279	0.7332	0.7791	0.7614	0.7278	0.7613
47	R.tenuicaulis	0.7051	0.6836	0.7193	0.6872	0.6843	0.6730	0.8175	0.7578	0.7847	0.7787	0.7181	0.6915
48	Genus "Z"	0.7465	0.7201	0.7363	0.6623	0.6785	0.7027	0.7819	0.6718	0.7378	0.7571	0.7281	0.7055

Similarity matrix from combined data with Gower's coefficient (flavonold data with simple matching coefficient)

	OTU no. ->	37	38	39	40	41	42	43	44	45	46	47	48
1	A.keriensis												
2	A.rupestris												
3	A.subrigida												
4	A.trinervis												
5	A.triplinervis												
6	C.aculeata												
7	C.fulvida												
8	C.leptophylla												
9	C.longifolia												
10	E.catipes												
11	E.meredithae												
12	E.planchonii												
13	E.sinclairii												
14	G.involucratum												
15	G.mackayi												
16	G.nitidulum												
17	G.traversii												
18	Ha.pulvinaris												
19	Ha.sinclairii												
20	He.backhousii												
21	He.bellidioides												
22	He.coralloides												
23	He.depressum												
24	He.dimorphumn												
25	He.dimorphums												
26	He.filicaule												
27	He.intermedium												
28	He.lanceolatum												
29	He.obcordatum												
30	He.parvifolium												
31	L.grandiceps												
32	L.leontopodium												
33	L."Marlborough"												
34	L."Peel"												
35	Pseudognaphalium												
36	Pterygopappus												
37	R.bryoides												
38	R.cinerea	0.8371											
39	R.eximia	0.9412	0.8250										
40	R.glabra	0.8472	0.8172	0.8350									
41	R.grandiflora	0.8317	0.7351	0.7729	0.8110								
42	R.hectori	0.9146	0.8206	0.9024	0.8325	0.8272							
43	R.hookeri	0.7447	0.7547	0.7812	0.8009	0.7850	0.8143						
44	R."L"	0.9518	0.8229	0.8916	0.8576	0.8517	0.9125	0.7785					
45	R."M"	0.8276	0.8423	0.8396	0.8127	0.7422	0.8232	0.7557	0.8133				
46	R.petriensis	0.8148	0.7954	0.8024	0.8615	0.8323	0.8619	0.8248	0.8250	0.8165			
47	R.tenuicaulis	0.7718	0.7817	0.8080	0.8277	0.8116	0.8666	0.9512	0.8056	0.7828	0.8516		
48	Genus "Z"	0.7475	0.7930	0.8092	0.7977	0.7356	0.7891	0.8369	0.7569	0.7750	0.7975	0.8389	